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(57) Abrégé/Abstract:
Streptococcus polypeptides and polynucleotides encoding them are disclosed. Said polypeptides may be useful vaccine components for the prophylaxis or therapy of streptococcus infection in animals. Also disclosed are recombinant methods of producing the protein antigens as well as diagnostic assays for detecting streptococcus bacterial infection.

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(54) Title: STREPTOCOCCUS ANTIGENS

(57) Abstract: Streptococcus polypeptides and polynucleotides encoding them are disclosed. Said polypeptides may be useful vaccine components for the prophylaxis or therapy of streptococcus infection in animals. Also disclosed are recombinant methods of producing the protein antigens as well as diagnostic assays for detecting streptococcus bacterial infection.

STREPTOCOCCUS ANTIGENS

FIELD OF THE INVENTION

The present invention is related to antigens, epitopes and antibodies directed to these epitopes, more particularly polypeptide antigens of streptococcus pneumoniae pathogen which may be useful for prophylaxis, diagnostic or treatment of streptococcal infection.

BACKGROUND OF THE INVENTION

S. pneumoniae is an important agent of disease in man especially among infants, the elderly and immunocompromised persons. It is a bacterium frequently isolated from patients with invasive diseases such as bacteraemia/septicaemia, pneumonia, meningitis with high morbidity and mortality throughout the world. Even with appropriate antibiotic therapy, pneumococcal infections still result in many deaths. Although the advent of antimicrobial drugs has reduced the overall mortality from pneumococcal disease, the presence of resistant pneumococcal organisms has become a major problem in the world today. Effective pneumococcal vaccines could have a major impact on the morbidity and mortality associated with S. pneumoniae disease. Such vaccines would also potentially be useful to prevent otitis media in infants and young children.

Efforts to develop a pneumococcal vaccine have generally concentrated on generating immune responses to the pneumococcal capsular polysaccharide. More than 80 pneumococcal capsular serotypes have been identified on the basis of antigenic differences. The currently available pneumococcal vaccine, comprising 23 capsular polysaccharides that most frequently caused disease, has significant shortcomings related primarily to the poor immunogenicity of some capsular polysaccharides, the diversity of the serotypes and the differences in the distribution of serotypes over time, geographic areas and age

groups. In particular, the failure of existing vaccines and capsular conjugate vaccines currently in development to protect young children against all serotypes spurres evaluation of other S. pneumoniae components. Although immunogenicity of capsular polysaccharides can be improved, serotype specificity will still represent a major limitation of polysaccharide-based vaccines. The use of a antigenically conserved immunogenic pneumococcal protein antigen, either by itself or in combination with additional components, offers the possibility of a protein-based pneumococcal vaccine.

PCT WO 98/18930 published May 7, 1998 entitled "*Streptococcus Pneumoniae* antigens and vaccines" describes certain polypeptides which are claimed to be antigenic. However, no biological activity of these polypeptides is reported. Similarly, no sequence conservation is reported, which is a necessary species common vaccine candidate.

PCT WO 00/39299 describes polypeptides and polynucleotides encoding these polypeptides. PCT WO 00/39299 demonstrates that polypeptides designated as BVH-3 and BVH-11 provide protection against fatal experimental infection with pneumococci.

Therefore there remains an unmet need for Streptococcus antigens that may be used as components for the prophylaxis, diagnostic and/or therapy of Streptococcus infection.

SUMMARY OF THE INVENTION

An isolated polynucleotide comprising a polynucleotide chosen from;

(a) a polynucleotide encoding a polypeptide having at least 70% identity to a second polypeptide chosen from: table A, B, D, E or H;

(b) a polynucleotide encoding a polypeptide having at least 95% identity to a second polypeptide chosen from: table A, B, D, E or H;

(c) a polynucleotide encoding a polypeptide having an amino sequence chosen from table A, B, D, E or H; or fragments, analogs or derivatives thereof;

(d) a polynucleotide encoding a polypeptide chosen from: table A, B, D, E or H;

(e) a polynucleotide encoding a polypeptide capable of generating antibodies having binding specificity for a polypeptide having a sequence chosen from: table A, B, D, E or H;

(f) a polynucleotide encoding an epitope bearing portion of a polypeptide chosen from table A, B, D, E or H; and

(g) a polynycleotide complementary to a polynucleotide in (a), (b), (c), (d),(e) or (f).

In other aspects, there are provided novel polypeptides encoded by polynucleotides of the invention, pharmaceutical or vaccine composition, vectors comprising polynucleotides of the invention operably linked to an expression control region, as well as host cells transfected with said vectors and methods of producing polypeptides comprising culturing said host cells under conditions suitable for expression.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is the DNA sequence of SP64 BVH-3 gene; SEQ ID NO: 1

Figure 2 is a DNA sequence containing the complete SP64 BVH-3 gene at nucleotides 1777 to 4896; SEQ ID NO: 2

Figure 3 is the DNA sequence of SP64 BVH-11 gene; SEQ ID NO: 3

Figure 4 is a DNA sequence containing the complete SP64 BVH-11 gene at nucleotides 45 to 2567; SEQ ID NO: 4

Figure 5 is a DNA sequence containing the complete SP64 BVH-11-2 gene at nucleotides 114 to 2630; SEQ ID NO: 5

Figure 6 is the amino acid sequence of SP64 BVH-3 polypeptide; SEQ ID NO: 6

Figure 7 is the amino acid sequence of SP64 BVH-11 polypeptide; SEQ ID NO: 7

Figure 8 is the amino acid sequence of SP64 BVH-11-2 polypeptide; SEQ ID NO: 8

Figure 9 is the DNA sequence of SP63 BVH-3 gene; SEQ ID NO:9

Figure 10 is the amino acid sequence of SP63 BVH-3 polypeptide; SEQ ID NO: 10

Figure 11 is the amino acid sequence of 4D4.9 polypeptide; SEQ ID NO: 11

Figure 12 is the amino acid sequence of 7G11.7 polypeptide; SEQ ID NO: 12

Figure 13 is the amino acid sequence of 7G11.9 polypeptide; SEQ ID NO: 13

Figure 14 is the amino acid sequence of 4D3.4 polypeptide; SEQ ID NO: 14

Figure 15 is the amino acid sequence of 8E3.1 polypeptide; SEQ ID NO: 15

Figure 16 is the amino acid sequence of 1G2.2 polypeptide; SEQ ID NO: 16

Figure 17 is the amino acid sequence of 10C12.7 polypeptide; SEQ ID NO: 17

Figure 18 is the amino acid sequence of 14F6.3 polypeptide; SEQ ID NO: 18

Figure 19 is the amino acid sequence of B12D8.2 polypeptide; SEQ ID NO: 19

Figure 20 is the amino acid sequence of 7F4.1 polypeptide; SEQ ID NO: 20

Figure 21 is the amino acid sequence of 10D7.5 polypeptide; SEQ ID NO: 21

Figure 22 is the amino acid sequence of 10G9.3 polypeptide, 10A2.2 polypeptide and B11B8.1 polypeptide; SEQ ID NO: 22

Figure 23 is the amino acid sequence of 11B8.4 polypeptide; SEQ ID NO: 23

Figure 24 is the amino acid sequence of Mab H11B-11B8 target epitope; SEQ ID 163

Figure 25 is a schematic representation of the BVH-3 gene as well as location of gene sequences coding for the full length and truncated polypeptides. The relationships between DNA fragments are shown with respect to each other.

Figure 26 is a schematic representation of the BVH-11 gene as well as location of gene sequences coding for the full length and truncated polypeptides. The relationships between DNA fragments are shown with respect to each other.

Figure 27 is a schematic representation of the BVH-11-2 gene as well as location of gene sequences coding for the full

length and truncated polypeptides. The relationships between DNA fragments are shown with respect to each other.

Figure 28 is a schematic representation of the BVH-3 protein and the location of internal and surface epitopes recognized by certain monoclonal antibodies.

Figure 29 is a schematic representation of the BVH-11-2 protein and the location of protective surface epitopes recognized by certain monoclonal antibodies.

Figure 30 is a map of plasmid pURV22.HIS. Kan^R, kanamycin-resistance coding region; cI857, bacteriophage λ cI857 temperature-sensitive repressor gene; lambda pL, bacteriophage λ transcription promotor; His-tag, 6-histidine coding region; terminator, T1 transcription terminator; ori, colE1 origin of replication.

Figure 31 depicts the comparison of the amino acid sequences of BVH-3M (sp64) and BVH-3 (Sp63) proteins by using the program Clustal W from MacVector sequence analysis software (version 6.5.3). Underneath the alignment, there is a consensus line where * and . characters indicate identical and similar amino acid residues, respectively.

Figure 32 depicts the comparison of the amino acid sequences of BVH-3, BVH-11 and BVH-11-2 proteins by using the program Clustal W from MacVector sequence analysis software (version 6.5.3). Underneath the alignment, there is a consensus line where * and . characters indicate identical and similar amino acid residues, respectively.

Figure 33 is the DNA sequence of the NEW43 gene (SEQ ID No 257).

Figure 34 is the deduced amino acid sequence of NEW43 polypeptide (SEQ ID No 258).

DETAILED DESCRIPTION OF THE INVENTION

It was determined that portions of the BVH-3 and BVH-11 polypeptides were internal. Other portions were not present in important strains such as encapsulated s.pneumonia causing disease strains. It would be advantageous to have a polypeptide that comprises a portion that is not internal. When large portions of a polypeptide are internal, these portions are not exposed on the bacteria. However, these portions can be very immunogenic in a recombinant polypeptide and will not confer protection against infections. It would also be advantageous to have a polypeptide that comprises a portion that is present in most strains.

The present invention is concerned with polypeptides in which undesired portions have been deleted and/or modified in order to obtain a specific immune response.

In accordance with the present invention, there are also provided polypeptides or polynucleotides encoding such polypeptides comprising protective domains.

Surprisingly, when the undesired portion of the polypeptides are deleted or modified, the polypeptides have desired biological properties. This is surprising in view of the fact that some of these portions were described as being epitope bearing portion in the patent application PCT WO 98/18930. In other publications such as PCT WO 00/37105, portions identified as histidine triad and coil coiled regions were said to be of importance. The present inventors have found that variants of the polypeptide BVH-3 and BVH-11 in which certain portions were deleted and/or modified and chimeras of these polypeptides have

biological properties and generate a specific immune response.

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 70% identity to a second polypeptide comprising a sequence as disclosed in the present application, the tables and figures.

In accordance with one aspect of the present invention, there is provided an isolated polynucleotide comprising a polynucleotide chosen from;

(a) a polynucleotide encoding a polypeptide having at least 70% identity to a second polypeptide chosen from: table B, E or H;
(b) a polynucleotide encoding a polypeptide having at least 95% identity to a second polypeptide chosen from: table B, E or H;
(c) a polynucleotide encoding a polypeptide having an amino sequence chosen from table B, E or H or fragments, analogs or derivatives thereof;
(d) a polynucleotide encoding a polypeptide chosen from: table B, E or H;
(e) a polynucleotide encoding a polypeptide capable of generating antibodies having binding specificity for a polypeptide having a sequence chosen from: table B, E or H,
(f) a polynucleotide encoding an epitope bearing portion of a polypeptide chosen from table B, E or H; and
(g) a polynycleotide complementary to a polynucleotide in (a), (b), (c), (d),(e) or (f).

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 70% identity to a second polypeptide comprising a sequence chosen from table A, B, D, E, G or H or fragments, analogues or derivatives thereof.

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 95% identity to a second polypeptide comprising a sequence chosen from table A, B, D, E, G or H or fragments, analogues or derivatives thereof.

According to one aspect, the present invention relates to polypeptides characterised by the amino acid sequence chosen from table A, B, D, E, G or H or fragments, analogues or derivatives thereof.

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 70% identity to a second polypeptide comprising a sequence chosen from table A, B, D, E, G or H.

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 95% identity to a second polypeptide comprising a sequence chosen from table A, B, D, E, G or H.

According to one aspect, the present invention relates to polypeptides characterised by the amino acid sequence chosen from table A, B, D, E, G or H.

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 70% identity to a second polypeptide comprising a sequence chosen from table B, E or H or fragments, analogues or derivatives thereof.

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 95% identity to a second polypeptide comprising a sequence chosen from B, E or H or fragments, analogues or derivatives

thereof.

According to one aspect, the present invention relates to polypeptides characterised by the amino acid sequence chosen from table B, E or H or fragments, analogues or derivatives thereof.

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 70% identity to a second polypeptide comprising a sequence chosen from table B, E or H.

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 95% identity to a second polypeptide comprising a sequence chosen from B, E or H.

According to one aspect, the present invention relates to polypeptides characterised by the amino acid sequence chosen from table B, E or H.

In accordance with the present invention, all nucleotides encoding polypeptides and chimeric polypeptides are within the scope of the present invention.

In a further embodiment, the polypeptides or chimeric polypeptides in accordance with the present invention are antigenic.

In a further embodiment, the polypeptides or chimeric polypeptides in accordance with the present invention are immunogenic.

In a further embodiment, the polypeptides or chimeric polypeptides in accordance with the present invention can elicit an immune response in an individual.

In a further embodiment, the present invention also relates to polypeptides which are able to raise antibodies having binding specificity to the polypeptides or chimeric polypeptides of the present invention as defined above.

In one embodiment, the polypeptides of table A (BVH-3) or table D (BVH-11) comprise at least one epitope bearing portion.

In a further embodiment, the fragments of the polypeptides of the present invention will comprise one or more epitope bearing portion identified in Table C and F. The fragment will comprises at least 15 contiguous amino acid of the polypeptide of table C and F. The fragment will comprises at least 20 contiguous amino acid of the polypeptide of table C and F.

In a further embodiment, the epitope bearing portion of the polypeptide of table A(BVH-3) comprises at least one polypeptide listed in Table C.

In a further embodiment, the epitope bearing portion of the polypeptide of table B(BVH-11) comprises at least one polypeptide listed in Table F.

An antibody that " has binding specificity" is an antibody that recognises and binds the selected polypeptide but which does not substantially recognise and bind other molecules in a sample, such as a biological sample. Specific binding can be measured using an ELISA assay in which the selected polypeptide is used as an antigen.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. All publications, patent applications, patents, and

other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

In accordance with the present invention, "protection" in the biological studies is defined by a significant increase in the survival curve, rate or period. Statistical analysis using the Log rank test to compare survival curves, and Fisher exact test to compare survival rates and numbers of days to death, respectively, might be useful to calculate P values and determine whether the difference between the two groups is statistically significant. P values of 0.05 are regarded as not significant.

As used herein, "fragments", "derivatives" or "analogues" of the polypeptides of the invention include those polypeptides in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably conserved) and which may be natural or unnatural. In one embodiment, derivatives and analogues of polypeptides of the invention will have about 70% identity with those sequences illustrated in the figures or fragments thereof. That is, 70% of the residues are the same. In a further embodiment, polypeptides will have greater than 75% homology. In a further embodiment, polypeptides will have greater than 80% homology. In a further embodiment, polypeptides will have greater than 85% homology. In a further embodiment, polypeptides will have greater than 90% homology. In a further embodiment, polypeptides will have greater than 95% homology. In a further embodiment, polypeptides will have greater than 99% homology. In a further embodiment, derivatives and analogues of polypeptides of the invention will have less than about 20 amino acid residue substitutions, modifications or deletions and more preferably less than 10.

Preferred substitutions are those known in the art as conserved i.e. the substituted residues share physical or chemical properties such as hydrophobicity, size, charge or functional groups.

The skilled person will appreciate that analogues or derivatives of the proteins or polypeptides of the invention will also find use in the context of the present invention, i.e. as antigenic/immunogenic material. Thus, for instance proteins or polypeptides which include one or more additions, deletions, substitutions or the like are encompassed by the present invention. In addition, it may be possible to replace one amino acid with another of similar "type". For instance replacing one hydrophobic amino acid with another hydrophilic amino acid.

One can use a program such as the CLUSTAL program to compare amino acid sequences. This program compares amino acid sequences and finds the optimal alignment by inserting spaces in either sequence as appropriate. It is possible to calculate amino acid identity or similarity (identity plus conservation of amino acid type) for an optimal alignment. A program like BLASTx will align the longest stretch of similar sequences and assign a value to the fit. It is thus possible to obtain a comparison where several regions of similarity are found, each having a different score. Both types of identity analysis are contemplated in the present invention.

In an alternative approach, the analogues or derivatives could be fusion proteins, incorporating moieties which render purification easier, for example by effectively tagging the desired protein or polypeptide, It may be necessary to remove the "tag" or it may be the case that the fusion protein itself retains sufficient antigenicity to be useful.

In an additional aspect of the invention there are provided antigenic/immunogenic fragments of the proteins or polypeptides of the invention, or of analogues or derivatives thereof.

The fragments of the present invention should include one or more such epitopic regions or be sufficiently similar to such regions to retain their antigenic/immunogenic properties. Thus, for fragments according to the present invention the degree of identity is perhaps irrelevant, since they may be 100% identical to a particular part of a protein or polypeptide, analogue or derivative as described herein. The key issue, once again, is that the fragment retains the antigenic/immunogenic properties.

Thus, what is important for analogues, derivatives and fragments is that they possess at least a degree of the antigenicity/immunogenic of the protein or polypeptide from which they are derived.

In accordance with the present invention, polypeptides of the invention include both polypeptides and chimeric polypeptides.

Also included are polypeptides which have fused thereto other compounds which alter the polypeptides biological or pharmacological properties i.e. polyethylene glycol (PEG) to increase half-life; leader or secretory amino acid sequences for ease of purification; prepro- and pro- sequences; and (poly)saccharides.

Furthermore, in those situations where amino acid regions are found to be polymorphic, it may be desirable to vary one or more particular amino acids to more effectively mimic the different epitopes of the different streptococcus strains.

Moreover, the polypeptides of the present invention can be

modified by terminal -NH_2 acylation (e.g. by acetylation, or thioglycolic acid amidation, terminal carboxy amidation, e.g. with ammonia or methylamine) to provide stability, increased hydrophobicity for linking or binding to a support or other molecule.

Also contemplated are hetero and homo polypeptide multimers of the polypeptide fragments, analogues and derivatives. These polymeric forms include, for example, one or more polypeptides that have been cross-linked with cross-linkers such as avidin/biotin, gluteraldehyde or dimethylsuperimidate. Such polymeric forms also include polypeptides containing two or more tandem or inverted contiguous sequences, produced from multicistronic mRNAs generated by recombinant DNA technology.

Preferably, a fragment, analogue or derivative of a polypeptide of the invention will comprise at least one antigenic region i.e. at least one epitope.

In order to achieve the formation of antigenic polymers (i.e. synthetic multimers), polypeptides may be utilised having bishaloacetyl groups, nitroarylhalides, or the like, where the reagents being specific for thio groups. Therefore, the link between two mercapto groups of the different peptides may be a single bond or may be composed of a linking group of at least two, typically at least four, and not more than 16, but usually not more than about 14 carbon atoms.

In a particular embodiment, polypeptide fragments, analogues and derivatives of the invention do not contain a methionine (Met) starting residue. Preferably, polypeptides will not incorporate a leader or secretory sequence (signal sequence). The signal portion of a polypeptide of the invention may be determined according to established molecular biological techniques. In general, the polypeptide of interest may be isolated from a streptococcus culture and subsequently

sequenced to determine the initial residue of the mature protein and therefore the sequence of the mature polypeptide.

According to another aspect, there are provided vaccine compositions comprising one or more streptococcus polypeptides of the invention in admixture with a pharmaceutically acceptable carrier diluent or adjuvant. Suitable adjuvants include oils i.e. Freund's complete or incomplete adjuvant; salts i.e. $AlK(SO_4)_2$, $AlNa(SO_4)_2$, $AlNH_4(SO_4)_2$, silica, kaolin, carbon polynucleotides i.e. poly IC and poly AU. Preferred adjuvants include QuilA and Alhydrogel. Vaccines of the invention may be administered parenterally by injection, rapid infusion, nasopharyngeal absorption, dermoabsorption, or bucal or oral. Pharmaceutically acceptable carriers also include tetanus toxoid.

The term vaccine is also meant to include antibodies. In accordance with the present invention, there is also provided the use of one or more antibodies having binding specificity for the polypeptides of the present invention for the treatment or prophylaxis of streptococcus infection and/or diseases and symptoms mediated by streptococcus infection.

Vaccine compositions of the invention are used for the treatment or prophylaxis of streptococcus infection and/or diseases and symptoms mediated by streptococcus infection as described in P.R. Murray (Ed, in chief),E.J. Baron, M.A. Pfaller, F.C. Tenover and R.H. Yolken. Manual of Clinical Microbiology, ASM Press, Washington, D.C. sixth edition, 1995, 1482p which are herein incorporated by reference. In one embodiment, vaccine compositions of the present invention are used for the treatment or prophylaxis of meningitis, otitis media, bacteremia or pneumonia. In one embodiment, vaccine compositions of the invention are used for the treatment or prophylaxis of *streptococcus* infection and/or diseases and symptoms mediated by *streptococcus* infection, in particular

S.pneumoniae, group A *streptococcus* (*pyogenes*), group B *streptococcus* (GBS or *agalactiae*), *dysgalactiae*, *uberis*, *nocardia* as well as *Staphylococcus aureus*. In a further embodiment, the streptococcus infection is S.pneumoniae.

In a particular embodiment, vaccines are administered to those individuals at risk of streptococcus infection such as infants, elderly and immunocompromised individuals.

As used in the present application, the term " individuals" include mammals. In a further embodiment, the mammal is human.

Vaccine compositions are preferably in unit dosage form of about 0.001 to 100 µg/kg (antigen/body weight) and more preferably 0.01 to 10 µg/kg and most preferably 0.1 to 1 µg/kg 1 to 3 times with an interval of about 1 to 6 week intervals between immunizations.

Vaccine compositions are preferably in unit dosage form of about 0.1 µg to 10 mg and more preferably 1µg to 1 mg and most preferably 10 to 100 µg 1 to 3 times with an interval of about 1 to 6 week intervals between immunizations.

According to another aspect, there are provided polynucleotides encoding polypeptides characterised by the amino acid sequence chosen from table A, B, D, E, G or H or fragments, analogues or derivatives thereof.

According to another aspect, there are provided polynucleotides encoding polypeptides characterised by the amino acid sequence chosen from table B, E or H or fragments, analogues or derivatives thereof.

In one embodiment, polynucleotides are those illustrated in table A, B, D, E, G or H which encodes polypeptides of the

invention.

In one embodiment, polynucleotides are those illustrated in table B, E or H which encodes polypeptides of the invention.

It will be appreciated that the polynucleotide sequences illustrated in the figures may be altered with degenerate codons yet still encode the polypeptides of the invention. Accordingly the present invention further provides polynucleotides which hybridise to the polynucleotide sequences herein above described (or the complement sequences thereof) having 50% identity between sequences. In one embodiment, at least 70% identity between sequences. In one embodiment, at least 75% identity between sequences. In one embodiment, at least 80% identity between sequences. In one embodiment, at least 85% identity between sequences. In one embodiment, at least 90% identity between sequences. In a further embodiment, polynucleotides are hybridizable under stringent conditions i.e. having at least 95% identity. In a further embodiment, more than 97% identity.

Suitable stringent conditions for hybridation can be readily determined by one of skilled in the art (see for example Sambrook et al., (1989) Molecular cloning : A Laboratory Manual, 2nd ed, Cold Spring Harbor, N.Y.; Current Protocols in Molecular Biology, (1999) Edited by Ausubel F.M. et al., John Wiley & Sons, Inc., N.Y.).

In a further embodiment, the present invention provides polynucleotides that hybridise under stringent conditions to either

(a) a DNA sequence encoding a polypeptide or

(b) the complement of a DNA sequence encoding a polypeptide;

wherein said polypeptide comprising a sequence chosen from table A, B, D, E, G or H or fragments or analogues thereof.

In a further embodiment, the present invention provides polynucleotides that hybridise under stringent conditions to either

(c) a DNA sequence encoding a polypeptide or

(d) the complement of a DNA sequence encoding a polypeptide;

wherein said polypeptide comprising a sequence chosen from table B, E or H or fragments or analogues thereof.

In a further embodiment, the present invention provides polynucleotides that hybridise under stringent conditions to either

(a) a DNA sequence encoding a polypeptide or

(b) the complement of a DNA sequence encoding a polypeptide;

wherein said polypeptide comprises at least 10 contiguous amino acid residues from a polypeptide comprising a sequence chosen from table A, B, D, E, G or H or fragments or analogues thereof.

In a further embodiment, the present invention provides polynucleotides that hybridise under stringent conditions to either

(c) a DNA sequence encoding a polypeptide or

(d) the complement of a DNA sequence encoding a polypeptide;

wherein said polypeptide comprises at least 10 contiguous amino acid residues from a polypeptide comprising a sequence chosen from table B, E or H or fragments or analogues thereof.

In a further embodiment, polynucleotides are those encoding polypeptides of the invention illustrated in table A, B, D, E, G or H.

As will be readily appreciated by one skilled in the art, polynucleotides include both DNA and RNA.

The present invention also includes polynucleotides complementary to the polynucleotides described in the present application.

In a further aspect, polynucleotides encoding polypeptides of the invention, or fragments, analogues or derivatives thereof, may be used in a DNA immunization method. That is, they can be incorporated into a vector which is replicable and expressible upon injection thereby producing the antigenic polypeptide in vivo. For example polynucleotides may be incorporated into a plasmid vector under the control of the CMV promoter which is functional in eukaryotic cells. Preferably the vector is injected intramuscularly.

According to another aspect, there is provided a process for producing polypeptides of the invention by recombinant techniques by expressing a polynucleotide encoding said polypeptide in a host cell and recovering the expressed polypeptide product. Alternatively, the polypeptides can be produced according to established synthetic chemical techniques i.e. solution phase or solid phase synthesis of oligopeptides which are ligated to produce the full polypeptide (block ligation).

General methods for obtention and evaluation of polynucleotides and polypeptides are described in the following references: Sambrook et al, Molecular Cloning: A Laboratory Manual, 2nd ed, Cold Spring Harbor, N.Y., 1989; Current Protocols in Molecular Biology, Edited by Ausubel F.M. et al., John Wiley and Sons, Inc. New York; PCR Cloning Protocols, from Molecular Cloning to Genetic Engineering, Edited by White B.A., Humana Press, Totowa, New Jersey, 1997, 490 pages; Protein Purification, Principles and Practices,

Scopes R.K., Springer-Verlag, New York, 3rd Edition, 1993, 380 pages; Current Protocols in Immunology, Edited by Coligan J.E. et al., John Wiley & Sons Inc., New York which are herein incorporated by reference.

For recombinant production, host cells are transfected with vectors which encode the polypeptide, and then cultured in a nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the genes. Suitable vectors are those that are viable and replicable in the chosen host and include chromosomal, non-chromosomal and synthetic DNA sequences e.g. bacterial plasmids, phage DNA, baculovirus, yeast plasmids, vectors derived from combinations of plasmids and phage DNA. The polypeptide sequence may be incorporated in the vector at the appropriate site using restriction enzymes such that it is operably linked to an expression control region comprising a promoter, ribosome binding site (consensus region or Shine-Dalgarno sequence), and optionally an operator (control element). One can select individual components of the expression control region that are appropriate for a given host and vector according to established molecular biology principles (Sambrook et al, Molecular Cloning: A Laboratory Manual, 2nd ed, Cold Spring Harbor, N.Y., 1989; Current Protocols in Molecular Biology, Edited by Ausubel F.M. et al., John Wiley and Sons, Inc. New York incorporated herein by reference). Suitable promoters include but are not limited to LTR or SV40 promoter, E.coli lac, tac or trp promoters and the phage lambda P_L promoter. Vectors will preferably incorporate an origin of replication as well as selection markers i.e. ampicilin resistance gene. Suitable bacterial vectors include pET, pQE70, pQE60, pQE-9, pbs, pD10 phagescript, psiX174, pbluescript SK, pbsks, pNH8A, pNH16a, pNH18A, pNH46A, ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 and eukaryotic vectors pBlueBacIII, pWLNEO, pSV2CAT, pOG44, pXT1, pSG, pSVK3, pBPV, pMSG and pSVL. Host cells may be bacterial i.e. E.coli, Bacillus subtilis, Streptomyces;

fungal i.e. Aspergillus niger, Aspergillus nidulins; yeast i.e. Saccharomyces or eukaryotic i.e. CHO, COS.

Upon expression of the polypeptide in culture, cells are typically harvested by centrifugation then disrupted by physical or chemical means (if the expressed polypeptide is not secreted into the media) and the resulting crude extract retained to isolate the polypeptide of interest. Purification of the polypeptide from culture media or lysate may be achieved by established techniques depending on the properties of the polypeptide i.e. using ammonium sulfate or ethanol precipitation , acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, hydroxylapatite chromatography and lectin chromatography. Final purification may be achieved using HPLC.

The polypeptide may be expressed with or without a leader or secretion sequence. In the former case the leader may be removed using post-translational processing (see US 4,431,739; US 4,425,437; and US 4,338,397 incorporated herein by reference) or be chemically removed subsequent to purifying the expressed polypeptide.

According to a further aspect, the streptococcus polypeptides of the invention may be used in a diagnostic test for streptococcus infection, in particular S. pneumoniae infection. Several diagnostic methods are possible, for example detecting streptococcus organism in a biological sample, the following procedure may be followed:

a) obtaining a biological sample from a patient;

b) incubating an antibody or fragment thereof reactive with a streptococcus polypeptide of the invention with the biological sample to form a mixture; and

c) detecting specifically bound antibody or bound fragment in the mixture which indicates the presence of streptococcus.

Alternatively, a method for the detection of antibody specific to a streptococcus antigen in a biological sample containing or suspected of containing said antibody may be performed as follows:

a) obtaining a biological sample from a patient;

b) incubating one or more streptococcus polypeptides of the invention or fragments thereof with the biological sample to form a mixture; and

c) detecting specifically bound antigen or bound fragment in the mixture which indicates the presence of antibody specific to streptococcus.

One of skill in the art will recognize that this diagnostic test may take several forms, including an immunological test such as an enzyme-linked immunosorbent assay (ELISA), a radioimmunoassay or a latex agglutination assay, essentially to determine whether antibodies specific for the polypeptide are present in an organism.

The DNA sequences encoding polypeptides of the invention may also be used to design DNA probes for use in detecting the presence of streptococcus in a biological sample suspected of containing such bacteria. The detection method of this invention comprises:

a) obtaining the biological sample from a patient;

b) incubating one or more DNA probes having a DNA sequence encoding a polypeptide of the invention or fragments thereof with the biological sample to form a mixture; and

c) detecting specifically bound DNA probe in the mixture which indicates the presence of streptococcus bacteria.

The DNA probes of this invention may also be used for detecting circulating streptococcus i.e. S.pneumoniae nucleic acids in a sample, for example using a polymerase chain reaction, as a method of diagnosing streptococcus infections.

The probe may be synthesized using conventional techniques and may be immobilized on a solid phase, or may be labelled with a detectable label. A preferred DNA probe for this application is an oligomer having a sequence complementary to at least about 6 contiguous nucleotides of the streptococcus pneumoniae polypeptides of the invention.

Another diagnostic method for the detection of streptococcus in a patient comprises:

a) labelling an antibody reactive with a polypeptide of the invention or fragment thereof with a detectable label;

b) administering the labelled antibody or labelled fragment to the patient; and

c) detecting specifically bound labelled antibody or labelled fragment in the patient which indicates the presence of streptococcus.

A further aspect of the invention is the use of the streptococcus polypeptides of the invention as immunogens for the production of specific antibodies for the diagnosis and in particular the treatment of streptococcus infection. Suitable antibodies may be determined using appropriate screening methods, for example by measuring the ability of a particular antibody to passively protect against streptococcus infection in a test model. One example of an animal model is the mouse model described in the examples herein. The antibody may be a whole antibody or an antigen-binding fragment thereof and may belong to any immunoglobulin class. The antibody or fragment may be of animal origin, specifically of mammalian origin and more specifically of murine, rat or human origin. It may be a natural antibody or a fragment thereof, or if desired, a recombinant antibody or antibody fragment. The term recombinant antibody or antibody fragment means antibody or antibody fragment which was produced using molecular biology techniques. The antibody or antibody fragments may be polyclonal, or preferably monoclonal. It may be specific for

a number of epitopes associated with the streptococcus pneumoniae polypeptides but is preferably specific for one.

A further aspect of the invention is the use of the antibodies directed to the streptococcus polypeptides of the invention for passive immunization. One could use the antibodies described in the present application. Suitable antibodies may be determined using appropriate screening methods, for example by measuring the ability of a particular antibody to passively protect against streptococcus infection in a test model. One example of an animal model is the mouse model described in the examples herein. The antibody may be a whole antibody or an antigen-binding fragment thereof and may belong to any immunoglobulin class. The antibody or fragment may be of animal origin, specifically of mammalian origin and more specifically of murine, rat or human origin. It may be a natural antibody or a fragment thereof, or if desired, a recombinant antibody or antibody fragment. The term recombinant antibody or antibody fragment means antibody or antibody fragment which was produced using molecular biology techniques. The antibody or antibody fragments may be polyclonal, or preferably monoclonal. It may be specific for a number of epitopes associated with the streptococcus pneumoniae polypeptides but is preferably specific for one.

The following are reference tables summarizing the sequences disclosed in the present application:

TABLE A, B and C Variants and Epitope of BVH-3-

Table A

Family	Polypeptide SEQ ID NO
BVH-3	
New 21	aa 396-1039 of SEQ ID. 6
New 25	aa 233-1039 of SEQ ID.6
New 40	aa 408-1039 of SEQ ID.6

TABLE B -

Family	Polypeptide SEQ ID NO
BVH-3	
NEW1-mut1**	235
NEW35A	236
NEW42	237
NEW49	238
NEW50	239
NEW51	240
NEW52	241
NEW53	242
NEW54	243
NEW55	244
NEW56	245
NEW56-mut2**	245
NEW56-mut3**	245
NEW57	246
NEW63	247
NEW64	248
NEW65	249
NEW66	250
NEW76	251
NEW105	252
NEW106	253
NEW107	254

** silent mutation, i.e. the polypeptide is the same as New1 or New 56

TABLE C- Epitopes of BVH-3

7G11.7	12
7G11.9	13
B12D8.2	19
7F4.1	20
14F6.3	18
4D3.4	14
10C12.7	17
8E3.1	15
1G2.2	16

TABLE D, E and F Variants and Epitope of BVH-11-

TABLE D-

Family	Polypeptide SEQ ID NO
BVH-11	
New19	aa 497-838 of Seq.ID 8
New24	aa 227-838 of Seq.ID 8

TABLE E-

Family	Polypeptide SEQ ID NO
BVH-11	
New 43	258
NEW60	293
NEW61	294
NEW62	295
NEW80	296
NEW81	297
NEW82	298
NEW83	299
NEW84	300
NEW85	301
NEW88D1	302
NEW88D2	303
NEW88	304

TABLE F- epitopes of BVH-11

10D7.5	21
10G9.3	22
B11B8.1	22
10A2.2	22
11b8.4	23
3A4.1	24

TABLE G and H Chimeras-

TABLE G

Family	Polypeptide SEQ ID NO

Chimeras with BVH-11 and BVH-3	
New17	M*-**NEW5**-G*P*-**NEW1**
New20	M*-**NEW1**-G*P*-**NEW5**
New26	M*-**NEW10**-G*P*-**NEW25**
New27	M*-**NEW19**-G*P*-**NEW25**
New28	M*-**NEW10**-G*P*-**NEW1**
New29	M*-**NEW5**-G*P*-**NEW25**
New30	M*-**NEW4**-G*P*-**NEW25**
New31	M*-**NEW4**-G*P*-**NEW1**
NEW32	M*-**NE19**-G*P*-**NEW1**

* OPTIONAL AMINO ACID

TABLE H

Family Chimeras with BVH-11 and BVH-3	Polypeptide SEQ ID NO.
VP 89	305
VP 90	306
VP 91	307
VP 92	308
VP 93	309
VP 94	310
VP 108	311
VP109	312
VP 110	313
VP 111	314
VP112	315
VP113	316
VP114	317
VP115	318
VP116	319
VP117	320
VP119	321
VP120	322
VP121	323
VP122	324
VP123	325
VP124	326

EXAMPLE 1

This example describes the bacterial strains, plasmids, PCR primers, recombinant proteins and hybridoma antibodies used herein.

S. pneumoniae SP64 (serogroup 6) and SP63 (serogroup 9) clinical isolates were provided by the Laboratoire de la Santé Publique du Québec, Sainte-Anne-de-Bellevue; Rx1 strain, a nonencapsulated derivative of the type 2 strain D39 and the type 3 strain WU2 were provided by David E. Briles from University of Alabama, Birmingham and the type 3 clinical isolate P4241 was provided by the Centre de Recherche en Infectiologie du Centre Hospitalier de l'Université Laval, Sainte-Foy. E. coli strains DH5α (Gibco BRL, Gaithesburg, MD); AD494 (λDE3) (Novagen, Madison, WI) and BL21 (λDE3) (Novagen) as well as plasmid superlinker pSL301 vector (Invitrogen, San Diego, CA); pCMV-GH vector (gift from Dr. Stephen A. Johnston, Department for Biochemistry, University of Texas, Dallas, Texas); pET32 and pET21 (Novagen) and pURV22.HIS expression vectors (Figure 30) were used in this study. The pURV22.HIS vector contains a cassette of the bacteriophage λ cI857 temperature-sensitive repressor gene from which the functional P_R promoter has been deleted. The inactivation of the cI857 repressor by a temperature increase from the range of 30-37°C to 37-42°C results in the induction of the gene under the control of promoter λPL. The PCR primers used for the generation of the recombinant plasmids had a restriction endonuclease site at the 5'end, thereby allowing directional cloning of the amplified product into the digested plasmid vector. The PCR oligonucleotide primers used are listed in the following Table 1. The location of the gene sequences coding for BVH-3, BVH-11 and BVH-11-2 gene products is summarized in the Figure 25, Figure 26 and Figure 27, respectively.

Table 1. List of PCR oligonucleotide primers

Primer	SEQ ID NO	Sequence 5' - 3'	Nucleotide position	Restriction sites
OCRR 479	25	cagtagatctgtgcctatgcact aaac	SEQ ID 1: 61-78 SEQ ID 9: 1-18	BglII
OCRR 480	26	gatctctagactactgctattcc ttacgctatg	SEQ ID 2: 4909-4887 SEQ ID 9: 2528-2519	XbaI
OCRR 497	27	atcactcgagcattacctggata atcctgt	SEQ ID 1: 1525-1506	XhoI
OCRR 498	28	ctgctaagcttatgaaagattta gat	SEQ ID 1: 1534-1548	HindIII
OCRR 499	29	gatactcgagctgctattcctta c	SEQ ID 2: 4906-4893	XhoI
HAMJ 172	30	gaatctcgagttaagctgctgct aattc	SEQ ID 1: 675-661	XhoI
HAMJ 247	31	gacgctcgagcgctatgaaatca gataaattc	SEQ ID 1: 3117-3096	XhoI
HAMJ 248	32	gacgctcgagggcattacctgga taatcctgttcatg	SEQ ID 1: 1527-1501	XhoI
HAMJ 249	33	cagtagatctcttcatcatttat tgaaaagagg	SEQ ID 2: 1749-1771	BglII
HAMJ 278	34	ttatttcttccatatggacttga cagaagagcaaattaag	SEQ ID 1: 1414-1437	NdeI
HAMJ 279	35	cgccaagcttcgctatgaaatca gataaattc	SEQ ID 1: 3117-3096	HindIII
HAMJ 280	36	cgccaagcttttccacaatataa gtcgattgatt	SEQ ID 1: 2400-2377	HindIII
HAMJ 281	37	ttatttcttccatatggaagtac ctatcttggaaaaagaa	SEQ ID 1: 2398-2421	NdeI
HAMJ 300	38	ttatttcttccatatggtgccta tgcactaaaccagc	SEQ ID 1: 62-82	NdeI

Primer	SEQ ID NO	Sequence 5' - 3'	Nucleotide position	Restriction sites
HAMJ 313	39	ataagaatgcggccgcttccaca atataagtcgattgatt	SEQ ID 1: 2400-2377	NotI
OCRR 487	40	cagtagatctgtgcttatgaact aggtttgc	SEQ ID 3: 58-79	BglII
OCRR 488	41	gatcaagcttgctgctaccttta cttactctc	SEQ ID 4: 2577-2556	HindIII
HAMJ 171	42	ctgagatatccgttatcgttcaa acc	SEQ ID 3: 1060-1075	EcoRV
HAMJ 251	43	ctgcaagcttttaaaggggaata atacg	SEQ ID 3: 1059-1045	HindIII
HAMJ 264	44	cagtagatctgcagaagccttcc tatctg	SEQ ID 3: 682-700	BglII
HAMJ 282	45	tcgccaagcttcgttatcgttca aaccattggg	SEQ ID 3: 1060-1081	HindIII
HAMJ 283	46	ataagaatgcggccgccttactc tcctttaataaagccaatagtt	SEQ ID 3: 2520-2492	NotI
HAMJ 284	47	catgccatggacattgatagtct cttgaaacagc	SEQ ID 3: 856-880	NcoI
HAMJ 285	48	cgccaagcttcttactctccttt aataaagccaatag	SEQ ID 3: 2520-2494	HindIII
HAMJ 286	49	cgacaagcttaacatggtcgcta gcgttacc	SEQ ID 3: 2139-2119 SEQ ID 5: 2210-2190	HindIII
HAMJ 287	50	cataccatgggcctttatgaggc acctaag	SEQ ID 3: 2014-2034	NcoI
HAMJ 288	51	cgacaagcttaagtaaatcttca gcctctctcag	SEQ ID 3: 2376-2353	HindIII
HAMJ 289	52	gataccatggctagcgaccatgt tcaaagaa	SEQ ID 3: 2125-2146	NcoI
HAMJ 290	53	cgccaagcttatcatccactaac ttgactttatcac	SEQ ID 3: 1533-1508	HindIII

Primer	SEQ ID NO	Sequence 5' - 3'	Nucleotide position	Restriction sites
HAMJ 291	54	cataccatggatattcttgcctt cttagctccg	SEQ ID 3: 1531-1554	NcoI
HAMJ 301	55	catgccatggtgcttatgaacta ggtttgc	SEQ ID 3: 59-79	NcoI
HAMJ 302	56	cgccaagctttagcgttaccaaa accattatc	SEQ ID 3: 2128-2107	HindIII
HAMJ 160	57	gtattagatctgttcctatgaac ttggtcgtcacca	SEQ ID 5: 172-196	BglII
HAMJ 186	58	cgcctctagactactgtatagga gccgg	SEQ ID 5: 2613-2630	XbaI
HAMJ 292	59	catgccatggaaaacatttcaag ccttttacgtg	SEQ ID 5: 925-948	NcoI
HAMJ 293	60	cgacaagcttctgtataggagcc ggttgactttc	SEQ ID 5: 2627-2604	HindIII
HAMJ 294	61	catgccatggttcgtaaaaataa ggcagaccaag	SEQ ID 5: 2209-2232	NcoI
HAMJ 297	62	catgccatggaagcctattggaa tgggaag	SEQ ID 5: 793-812	NcoI
HAMJ 352	63	catgccatggaagcctattggaa tgggaagc	SEQ ID 5: 793-813	NcoI
HAMJ 353	64	cgccaagcttgtaggtaatttgc gcatttgg	SEQ ID 5: 1673-1653	HindIII
HAMJ 354	65	cgccaagcttctgtataggagcc ggttgac	SEQ ID 5: 2627-2608	HindIII
HAMJ 355	66	catgccatggatattcttgcctt cttagctcc	SEQ ID 5: 1603-1624	NcoI
HAMJ 404	67	ttatttcttccatatgcatggtg atcatttccattaca	SEQ ID 1: 1186-1207	NdeI
HAMJ 464	68	gatgcatatgaatatgcaaccga gtcagttaagc	SEQ ID 1: 697-720	NdeI
HAMJ 465	69	gatgctcgagagcatcaaatccg tatccatc	SEQ ID 1: 1338-1318	XhoI

Primer	SEQ ID NO	Sequence 5' - 3'	Nucleotide position	Restriction sites
HAMJ 466	70	gatgcatatggatcatttccatt acattcca	SEQ ID 1: 1192-1212	NdeI
HAMJ 467	71	gacaagcttggcattacctggat aatcctg	SEQ ID 1: 1527-1507	HindIII
HAMJ 352	72	catgccatggaagcctattggaa tgggaagc	SEQ ID 5: 793-813	NcoI
HAMJ 470	73	ataagaatgcggccgccgctatg aaatcagataaattc	SEQ ID 1: 3096-3117	NotI
HAMJ 471	168	atatgggcccctgtataggagcc ggttgactttc	SEQ ID 5: 2626-2604	Apa I
HAMJ 472	169	atatgggcccaatatgcaaccga gtcagttaagc	SEQ ID 1: 720-697	Apa I
HAMJ 350	170	atatgggcccaacatggtcgcta gcgttacc	SEQ ID 3: 2139-2119	Apa I
HAMJ 351	171	tcccgggcccgacttgacagaag agcaaattaag	SEQ ID 1: 1414-1437	Apa I
HAMJ 358	172	catgccatgggacttgacagaag agcaaattaag	SEQ ID 1: 1415-1437	NcoI
HAMJ 359	173	tcccgggccccgctatgaaatca gataaattc	SEQ ID 1: 3116-3096	Apa I
HAMJ 403	174	atatgggcccgacattgatagtc tcttgaaacagc	SEQ ID 3: 856-880	Apa I
HAMJ 361	175	cgccaagcttaacatggtcgcta gcgttacc	SEQ ID 3: 2139-2119	Hind III
HAMJ 483	176	atatgggcccttactctccttt aataaagccaatag	SEQ ID 3: 2520-2494	Apa I

Molecular biology techniques were performed according to standard methods. See for example, Sambrook, J., Fritsch, E.F., Maniatis, T., "Molecular cloning. A laboratory manual" Vol.1-2-3 (second edition) Cold Spring Harbour Laboratory Press, 1989, New York, which is herein incorporated by reference. PCR-amplified products were digested with restriction endonucleases and ligated to either linearized plasmid pSL301, pCMV-GH, pET or pURV22.HIS expression vector digested likewise or digested with enzymes that produce compatible cohesive ends. Recombinant pSL301 and recombinant pCMV-GH plasmids were digested with restriction enzymes for the in-frame cloning in pET expression vector. When pET

vectors were used, clones were first stabilized in E. coli DH5α before introduction into E. coli BL21(λDE3) or AD494 (λDE3) for expression of full-length or truncated BVH-3, BVH-11 or BVH-11-2 molecules. Each of the resultant plasmid constructs was confirmed by nucleotide sequence analysis. The recombinant proteins were expressed as N-terminal fusions with the thioredoxin and His-tag (pET32 expression system); as C-terminal fusions with an His-tag (pET21 expression system); or as N-terminal fusions with an His-tag (pURV22.HIS expression system). The expressed recombinant proteins were purified from supernatant fractions obtained after centrifugation of sonicated IPTG- (pET systems) or heat- (pURV22.HIS) induced E. coli using a His-Bind metal chelation resin (QIAgen, Chatsworth, CA). The gene products generated from S. pneumoniae SP64 are listed in the following Table 2. The gene fragment encoding BVH-3-Sp63 protein (amino acid residues 21 to 840 on SEQ ID NO: 10) was generated from S. pneumoniae SP63 using the PCR-primer sets OCRR479-OCRR480 and the cloning vector pSL301. The recombinant pSL301-BVH-3Sp63 was digested for the in-frame cloning in pET32 vector for the expression of the BVH-3-Sp63 molecule.

Table 2. Lists of truncated BVH-3, BVH-11, BVH-11-2 and Chimeric gene products generated from S. pneumoniae SP64

PCR-primer sets	Protein designation	Identification	Encoded amino acids (SEQ ID No6)	Cloning vector
OCRR479-OCRR480	BVH-3M	BVH-3 w/o ss	21-1039	pSL301
OCRR479-OCRR497	BVH-3AD	BVH-3 N'end w/o ss	21-509	pSL301
HAMJ248-HAMJ249	L-BVH-3AD	BVH-3 N'end	1-509	pET-21(+)
OCRR498-OCRR499	BVH-3B	BVH-3 C'end	512-1039	pSL301
OCRR479-HAMJ172	BVH-3C	BVH-3 N'end w/o ss	21-225	pET-32c(+)
OCRR487-OCRR488	BVH-11M	BVH-11 w/o ss	20-840	pCMV-GH
HAMJ251-OCRR487	BVH-11A	BVH-11 N'end w/o ss	20-353	pET-32c(+)

HAMJ171-OCRR488	BVH-	BVH-11 C'end	354-840	pET-32 a(+)
HAMJ264-OCRR488	BVH-	BVH-11 C'end	228-840	pET-32 a(+)
HAMJ278-HAMJ279	NEW1	BVH-3 C'end	472-1039	pET-21b(+)
HAMJ278-HAMJ280	NEW2	BVH-3 C'end	472-800	pET-21b(+)
HAMJ281-HAMJ279	NEW3	BVH-3 C'end	800-1039	pET-21b(+)
HAMJ284-HAMJ285	NEW4	BVH-11 C'end	286-840	pET-21d(+)
HAMJ284-HAMJ286	NEW5	BVH-11	286-713	pET-21d(+)
HAMJ287-HAMJ288	NEW6	BVH-11	672-792	pET-21d(+)
HAMJ285-HAMJ289	NEW7	BVH-11 C'end	709-840	pET-21d(+)
HAMJ284-HAMJ290	NEW8	BVH-11	286-511	pET-21d(+)
HAMJ286-HAMJ291	NEW9	BVH-11	511-713	pET-21d(+)
HAMJ160-HAMJ186	BVH-	BVH-11-2 w/o	20-838	pSL301
HAMJ292-HAMJ293	NEW10	BVH-11-2	271-838	pET-21d(+)
HAMJ293-HAMJ294	NEW11	BVH-11-2	699-838	pET-21d(+)
HAMJ282-HAMJ283	NEW13	BVH-11 C'end	354-840	pET-21b(+)
HAMJ286-HAMJ297	NEW14	BVH-11-2	227-699	pET-21d(+)
HAMJ300-HAMJ313	NEW15	BVH-3 N'end	21-800	pET-21b(+)
HAMJ301-HAMJ302	NEW16	BVH-11 N'end w/o ss	20-709	pET-21d(+)
HAMJ352-HAMJ353	NEW18	BVH-11-2 internal	227-520	pET21d(+)
HAMJ354-HAMJ355	NEW19	BVH-11-2 C'end	497-838	pET21d(+)
HAMJ404-HAMJ279	NEW21	BVH-3 C'end	396-1039	pET21b(+)
HAMJ464-HAMJ465	NEW22	BVH-3 internal	233-446	pET-21a(+)
HAMJ466-HAMJ467	NEW23	BVH-3 internal	398-509	pET-21b(+)
HAMJ352-HAMJ293	NEW24	BVH-11-2 C'end	227-838	pET-21d(+)
HAMJ464-HAMJ470	NEW25	BVH-3 C'end	233-1039	pET-21b(+)
HAMJ278-HAMJ279 (NEW 1) HAMJ282-HAMJ283 (NEW 13)	NEW12	Chimera*	M-NEW 1 -KL - NEW 13	pET 21 b (+)
HAMJ284-HAMJ350 (NEW 5) HAMJ351-HAMJ279 (NEW 1)	NEW17	Chimera*	M- NEW 5 -GP - NEW 1	pET 21 d (+)
HAMJ358-HAMJ359 (NEW 1) HAMJ403-	NEW20	Chimera*	M- NEW 1 -GP - NEW 5	pET 21 d (+)

HAMJ361(NEW5)				
HAMJ292-HAMJ471 (NEW 10) HAMJ472-HAMJ470(NEW25)	NEW26	Chimera*	M- NEW 10 -GP - NEW25	pET21d(+)
HAMJ355-HAMJ471 (NEW 19) HAMJ472-HAMJ470(NEW25)	NEW27	Chimera*	M- NEW 19 -GP - NEW25	pET21d(+)
HAMJ292-HAMJ471 (NEW 10) HAMJ351 - HAMJ279(NEW1)	NEW28	Chimera*	M- NEW 10 -GP - NEW1	pET21d(+)
HAMJ284-HAMJ350 (NEW 5) HAMJ472-HAMJ470(NEW25)	NEW29	Chimera*	M- NEW 5 -GP - NEW25	pET21d(+)
HAMJ284-HAMJ483 (NEW 4) HAMJ472-HAMJ470(NEW25)	NEW30	Chimera*	M- NEW 4 -GP - NEW25	pET21d(+)
HAMJ284-HAMJ483 (NEW 4) HAMJ351-HAMJ279(NEW1)	NEW31	Chimera*	M- NEW 4 -GP - NEW1	pET21d(+)
HAMJ355-HAMJ471 (NEW 19) HAMJ351-HAMJ279(NEW1)	NEW32	Chimera*	M- NEW 19 -GP - NEW1	pET21d(+)

w/o ss : without signal sequence. Analysis of the BVH-3, BVH-11 and BVH-11-2 protein sequences suggested the presence of putative hydrophobic leader sequences.

* encoded amino acids for the chimeras are expressed as the gene product, additional non essential amino acids residue were added M is methionine, K is lysine, L is leucine, G is glycine and P is proline.

Monoclonal antibody (Mab)-secreting hybridomas were obtained by fusions of spleen cells from immunized mice and non-secreting, HGPRT-deficient mouse myeloma SP2/0 cells by the methods of Fazekas De St-Groth and Scheidegger (J Immunol Methods 35 : 1-21, 1980) with modifications (J. Hamel et al. J Med Microbiol 23 : 163-170, 1987). Female BALB/c mice (Charles River, St-Constant, Quebec, Canada) were immunized with either BVH-3M (thioredoxin-His•Tag-BVH-3M fusion protein/ pET32

system), BVH-11M (thioredoxin-His•Tag-BVH-11M fusion protein/ pET32 system), BVH-11-2M (thioredoxin-His•Tag-BVH-11-2M fusion protein/ pET32 system), BVH-11B (thioredoxin-His•Tag-BVH-11B fusion protein/ pET32 system), BVH-3M (His•Tag-BVH-3 fusion protein/ pURV22.HIS system) or NEW1 (NEW1-His•Tag fusion protein/ pET21 system) gene products from S. pneumoniae strain SP64 to generate the Mab series H3-, H11-, H112-, H11B-, H3V-, and HN1-, respectively. Culture supernatants of hybridomas were initially screened by enzyme-linked-immunoassay (ELISA) according to the procedure described by Hamel et al. (Supra) using plates coated with preparations of purified recombinant BVH-3, BVH-11 and/or BVH-11-2 proteins or suspensions of heat-killed S. pneumoniae cells. The Mab-secreting hybridomas selected for further characterization are listed in Table 3 and Table 4 from the following Example 2. The class and subclass of Mab immunoglobulins were determined by ELISA using commercially available reagents (Southern Biotechnology Associates, Birmingham, AL).

Furthermore, the cloning and expression of chimeric gene(s) encoding for chimeric polypeptides and the protection observed after vaccination with these chimeric polypeptides are described.

BVH-3 and BVH-11 gene fragments corresponding to the 3'end of the genes were amplified by PCR using pairs of oligonucleotides engineered to amplify gene fragments to be included in the chimeric genes. The primers used had a restriction endonuclease site at the 5' end, thereby allowing directional in-frame cloning of the amplified product into digested plasmid vectors (Table 1 and Table 2). PCR-amplified products were digested with restriction endonucleases and ligated to linearized plasmid pET21 or pSL301 vector. The resultant plasmid constructs were confirmed by nucleotide sequence analysis. The recombinant pET21 plasmids containing

a PCR product were linearized by digestion with restriction enzymes for the in-frame cloning of a second DNA fragment and the generation of a chimeric gene encoding for a chimeric pneumococcal protein molecule. Recombinant pSL301 plasmids containing a PCR product were digested with restriction enzymes for the obtention of the DNA inserts. The resulting insert DNA fragments were purified and inserts corresponding to a given chimeric gene were ligated into pET21 vector for the generation of a chimeric gene. The recombinant chimeric polypeptides listed in Table 2 were as C-terminal fusion with an His-tag. The expressed recombinant proteins were purified from supernatant fractions obtained from centrifugation of sonicated IPTG-induced E. coli cultures using a His-Bind metal chelation resin (QIAgen, Chatsworth, CA).

Groups of 8 female BALB/c mice (Charles River) were immunized subcutaneously two times at three-week intervals with 25 μg of either affinity purified His•Tag-fusion protein identifed in presence of 15-20 μg of QuilA adjuvant. Ten to 14 days following the last immunization, the mice were challenged challenged intravenously
with 10E5-10E6 CFU of S. pneumoniae type 3 strain WU2. The polypeptides and fragments are capable of eliciting a protective immune response.

Table 2A

Experiment	Immunogen	Alive : Dead	Days to death post-infection
1	none	0 : 8	1, 1, 1, 1, 1, 1, 1, 1
	NEW 1	2 : 6	1, 2, 2, 2, 2, 2, >14, >14
	NEW 13	1 : 7	1, 1, 3, 3, 4, 5, 5, >14
	NEW 12	6 : 2	3, 11, 6X >14
	BVH-3M	1 : 7	3, 3, 3, 3, 3, 3, 3, >14
2	none	0 : 8	1, 1, 1, 1, 1, 1, 1,

			1
	NEW 17	7 : 1	4, 7 X >14
	NEW 12	3 : 5	3, 3, 3, 4, 5, >14, >14, >14
3	none	0 : 8	2, 2, 2, 2, 2, 2, 2, 2
	NEW 18	1 : 7	2, 2, 2, 2, 3, 3, 3, 3
	NEW 19	8 : 0	8 X >14
	NEW 10	8 : 0	8 X >14
	BVH-11-2	8 : 0	8 X >14

EXAMPLE 2

This example describes the identification of peptide domains carrying target epitopes using Mabs and recombinant truncated proteins described in example 1.

Hybridomas were tested by ELISA against truncated BVH-3, BVH-11 or BVH-11-2 gene products in order to characterize the epitopes recognized by the Mabs. The truncated gene products were generated from S. pneumoniae SP64 strain except for BVH-3-Sp63 which was generated from S. pneumoniae SP63 strain. As a positive control, the reactivity of each antibody was examined with full-length BVH-3, BVH-11 or BVH-11-2 recombinant proteins. In some cases, the Mab reactivity was evaluated by Western immunoblotting after separation of the gene product by SDS-PAGE and transfer on nitrocellulose paper. The reactivities observed is set forth in the following Table 3 and Table 4.

Table 3. ELISA reactivity of BVH-3-reactive Mabs with a panel of eleven BVH-3 gene products and the BVH-11M molecule

	Gene products tested											
Mabs (IgG isotype)	BVH-3M	BVH-3AD	BVH-3B	BVH-3C	NEW 1	NEW 2	NEW 3	NEW 21	NEW 22	NEW 23	BVH-3 Sp63	BVH-11M
H3-4F9 (1)	+	+	-	+	-	-	-	-	-	-	+	+
H3-4D4 (1)	+	+	-	+	-	-	-	-	-	-	+	+
H3-9H12 (1)	+	+	-	+	-	-	-	-	-	-	+	+
H3-7G2 (1)	+	+	-	-	-	-	-	-	+	-	-	-
H3-10A1 (1)	+	+	-	-	-	-	-	+	-	+	+	-
H3-4D3 (1)	+	-	+	-	+	-	+	+	-	-	+	-
H11-6E7 (1)	+	+	-	+	-	-	-	NT	NT	NT	+	+
H11-10H10 (2a)	+	+	-	+	-	-	-	NT	NT	NT	+	+
H11-7G11 (2b)	+	+	+	+	+	+	-	NT	NT	NT	+	+
H3V-4F3 (1)	+	-	+	-	+	-	-	+	-	-	+	-
H3V-2F2 (1)	+	-	+	-	+	+	-	+	-	-	+	-
H3V-7F4 (1)	+	-	+	-	+	+	-	+	-	-	+	-
H3V-7H3 (1)	+	-	+	-	+	-	+	+	-	-	+	-

	Gene products tested											
Mabs (IgG isotype)	BVH-3M	BVH-3AD	BVH-3B	BVH-3C	NEW 1	NEW 2	NEW 3	NEW 21	NEW 22	NEW 23	BVH-3 Sp63	BVH-11M
H3V-13B8 (1)	+	-	+	-	+	-	+	+	-	-	+	-
H3V-9C2 (1)	+	+	-	+/-	-	-	-	-	+	-	+/-	+/-
H3V-9C6 (1)	+	+	-	-	-	-	-	-	+	-	-	-
H3V-16A7 (1)	+	+	-	-	-	-	-	+	-	+	-	-
H3V-15A10 (1)	+	+	+	+/-	+	+	-	+	+	+	+	+/-
H3V-6B3 (1/2)	+	+	NT	NT	+	+	-	+	+	-	NT	-
HN1-5H3 (2b)	+	-	+	NT	+	-	-	+	-	-	+	-
HN1-8E3 (2a)	+	-	+	NT	+	-	-	+	-	-	+	-
HN1-14F6 (2a)	+	-	+	NT	+	-	-	+	-	-	+	-
HN1-2G2 (1)	+	-	+	NT	+	+	-	+	-	-	+	-

	Gene products tested											
Mabs (IgG isotype)	BVH-3M	BVH-3AD	BVH-3B	BVH-3C	NEW 1	NEW 2	NEW 3	NEW 21	NEW 22	NEW 23	BVH-3 Sp63	BVH-11M
HN1-12D8 (2a)	+	-	+	NT	+	+	-	+	-	-	+	-
HN1-14B2 (2a)	+	-	+	NT	+	+	-	+	-	-	+	-
HN1-1G2 (2a)	+	-	+	NT	+	-	+	+	-	-	+	-
HN1-10C12 (1)	+	-	+	NT	+	-	+	+	-	-	+	-
HN1-3E5 (1)	+	+	-	-	+	+	-	+	-	+	+	-

NT : not tested

+/- : very low reactivity but higher than background, possible non-specific Mab binding

Table 4. ELISA reactivity of BVH-11 and/or BVH-11-2-reactive Mabs with a panel of fourteen BVH-11 and BVH-11-2 gene products and the BVH-3M molecule

	Gene products tested															
Mabs (IgG isotype)	BVH-11M	BVH-11A	BVH-11B	BVH-11C	NEW 5	NEW 6	NEW 7	NEW 8	NEW 9	NEW 10	NEW 11	NEW 14	NEW 18	NEW 19	BVH-11-2-M	BVH-3M
H3-4F9 (1)	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+
H3-4D4 (1)	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+
H3-9H12 (1)	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+
H11-6E7 (1)	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+
H11-10H10 (2a)	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+
H11-7G11 (2b)	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+
H11-1B12 (1)	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-
H11-7B9	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-

Mabs (IgG isotype)	Gene products tested														
	BVH-11M	BVH-11A	BVH-11B	BVH-11C	NEW 5	NEW 6	NEW 7	NEW 8	NEW 9	NEW 10	NEW 11	NEW 14	NEW 18	NEW 19	BVH-11-2-M	BVH-3M
(2a)																
H11-3H5 (1)	+	-	+	+	+	-	-	-*	-	+	-	+	+	-	+	-
H11-10B8 (1)	+	-	+	+	+	-	-	-*	-	+	-	+	+	-	+	-
H11-1A2 (1)	+	-	+	+	+	-	-	-*	-	+	-	+	+	-	+	-
H112-3A1 (1)	+	-	+	NT	+	-	-	+	-	+	-	+	+	-	+	-
H112-13C11 (1)	+	+/-	+	NT	+	-	-	+	-	+	-	+	+	-	+	-
H112-10H10 (1)	+	+	-	NT	+	-	-	+	-	+	-	+	+	-	+	-
H112-1D8 (2a)	+	+	-	NT	+	-	-	+	-	+	-	+	+	-	+	-

	Gene products tested															
Mabs (IgG isotype)	BVH-11M	BVH-11A	BVH-11B	BVH-11C	NEW 5	NEW 6	NEW 7	NEW 8	NEW 9	NEW 10	NEW 11	NEW 14	NEW 18	NEW 19	BVH-11-2-M	BVH-3M
H112-10G9 (2b)	+	-	+	NT	+	-	-	-	+	+	-	+	-	+	+	-
H112-10A2 (1)	+	-	+	NT	+	-	-	+/-	+	+	-	+	-	+	+	-
H112-3E8 (2a)	+	-	+	NT	+	-	-	+/-	-	+	-	+	-	+	+	-
H112-10D7 (2a)	+	-	+	NT	+	-	-	-	-	+	-	+	-	-	+	-
H112-2H7 (2a)	+	+	-	NT	-	-	-	-	-	-	-	-	-	-	+	-
H112-6H7 (1)	+	+	-	NT	-	-	-	-	-	-	-	-	-	-	+	-
H112-3A4 (2a)	-	-	-	NT	-	-	-	-	-	+	+	-	-	+	+	-
H112-	-	-	-	NT	-	-	-	-	-	+	+	-	-	+	+	-

Mabs (IgG isotype)	Gene products tested														
	BVH-11M	BVH-11A	BVH-11B	BVH-11C	NEW 5	NEW 6	NEW 7	NEW 8	NEW 9	NEW 10	NEW 11	NEW 14	NEW 18	NEW 19	BVH-11-2-M	BVH-3M
10C5 (1)																
H112-14H6 (1)	-	-	-	NT	-	-	-	-	-	+	+	-	-	+	+	-
H112-7G2 (1)	-	-	-	NT	-	-	-	-	-	+	-	+	+	-	+	-
H112-13H10 (2a)	-	-	-	NT	-	-	-	-	-	-	-	+	+	-	+	-
H112-7E8 (2b)	+/-	-	-	NT	-	-	-	-	-	-	-	-	+/-	-	+	-
H112-7H6 (1)	+/-	-	-	NT	-	-	-	-	-	+/-	-	-	-	-	+	-
H11B-5F10 (1)	+	-	+	+	+	-	-	+	-	+	-	+	+	-	+	-
H11B-15G2 (1)	+	-	+	+	+	-	-	+	-	+	-	+	+	-	+	-
H11B-	+	-	+	+	+	-	-	-	+	+	-	+	-	+	+	-

	Gene products tested															
Mabs (IgG isotype)	BVH-11M	BVH-11A	BVH-11B	BVH-11C	NEW 5	NEW 6	NEW 7	NEW 8	NEW 9	NEW 10	NEW 11	NEW 14	NEW 18	NEW 19	BVH-11-2-M	BVH-3M
13D5 (2)																
H11B-11B8 (1)	+	-	+	+	+	-	-	-	+	+	-	+	-	+	+	-
H11B-7E11 (1)	+	-	+	+	+	-	-	-	-	+	-	+	-	-	+	-
H11B-1C9 (1)	+	-	+	+	+	-	-	-	-	+	-	+	-	-	+	-
H11B-5E3 (2)	+	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-
H11B-6E8 (1)	+	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-

NT : not tested

+/- : very low reactivity but higher than background, possible non-specific Mab binding

* : a strong signal was detected when tested by Western immunoblotting

The deduced locations of the epitopes are summarized in Figure 28 and Figure 29. As can be seen from the data in Table 3, BVH-3-reactive Mabs can be divided into two groups : BVH-3A- and BVH-3B-reactive Mabs with the exception of Mabs H11-7G11 and H3V-15A10 which reacted with both, BVH-3A and BVH-3B molecules. The BVH-3A-reactive Mabs can be subdivided in two subgroups of antibodies depending of their reactivity or lack of reactivity with BVH-3C recombinant protein. Mab reactive with BVH-3C protein recognized epitopes shared by both, BVH-3 and BVH-11 proteins. As can be seen in Table 4, these BVH-3- and BVH-11-cross-reactive Mabs were also reactive with BVH-11A and BVH-11-2M recombinant proteins. BVH-3B-reactive Mabs can be subdivided into three subgroups according to their reactivity with NEW1, NEW2 and NEW3 recombinant proteins. Some Mabs were only reactive with the NEW1 protein while other Mabs were reactive with either, NEW1 and NEW2 or NEW1 and NEW3 recombinant proteins.

Mabs H11-7G11 and H3V-15A10 react with epitopes in more than one position on BVH-3. The reactivity of H11-7G11 with BVH-3AD, BVH-3B, BVH-3C, BVH-11A and BVH-11-2M molecules suggests that H11-7G11 epitope might comprised HXXHXH sequence. This sequence is repeated, respectively, 6 and 5 times in BVH-3 and BVH-11/BVH-11-2 protein sequences. The lack of reactivity of Mab H11-7G11 with NEW 10 molecule suggests that the epitope includes the HGDHXH sequence. Multiple-position mapping of H3V-15A10 epitope on BVH-3 is suggested by the reactivity of the Mab with two BVH-3 fragments that do not overlap.

Interestingly, Mabs H3-7G2, H3V-9C6 and H3V-16A7 were not reactive with BVH-3 Sp63 thus allowing the location of their corresponding epitopes on a 177-amino acid fragment comprised between amino acids 244 and 420 on BVH-3 molecule of S. pneumoniae SP64 (Figure 31).

As can be seen from the data in Table 4, the Mabs that are reactive with BVH-11- and/or BVH-11-2 and that do not recognize BVH-3 molecules can be divided into three groups according to their reactivities with BVH-11A and NEW10 recombinant proteins. Some Mabs reacted exclusively with either BVH-11A or NEW10 protein while other Mabs were reactive with both, BVH-11A and NEW10 recombinant proteins.

EXAMPLE 3

This example describes the construction of BVH-3 and BVH-11-2 gene libraries for the mapping of epitopes.

BVH-3 and BVH-11-2 gene libraries were constructed using recombinant pCMV-GH and PSL301 plasmid DNA containing respectively, BVH-3 gene sequence spanning nucleotides 1837 to 4909 (SEQ ID NO: 2) or BVH-11-2 gene sequence spanning nucleotides 172 to 2630 (SEQ ID NO: 5) and the Novatope® library construction and screening system (Novagen). The recombinant plasmids containing BVH-3 or BVH-11-2 gene fragment were purified using QIAgen kit (Chatsworth, CA) and digested with the restriction enzymes BglII and XbaI respectively. The resulting BglII-XbaI DNA fragments were purified using the QIAquick gel extraction kit from QIAgen and digested with Dnase I for the generation of randomly cleaved DNA. DNA fragments of 50 to 200 bp were purified, treated with T4 DNA polymerase to blunt the target DNA ends and add a single 3'dA residue, and ligated into pSCREEN-T-Vector (Novagen) following the procedures suggested by the manufacturer (Novatope® System, Novagen). The gene libraries of E. coli clones, each of which expressing a small peptide derived from BVH-3 or BVH-11-2 genes were screened by standard colony lift methods using Mabs as immunoprobes. The colony screening was not successful with Mabs producing very high backgrounds on colony lifts. Moreover, in some cases, Mabs

failed to detect epitope-expressing-colonies. The lack of reactivity can possibly be explained by the small amount of recombinant proteins produced or the recognition of conformation-dependent epitopes consisting of different protein domains. Sequencing of DNA inserts from positive clones determined the location of the segment that encodes the target epitope. The data are presented in Table 5. The peptides encoded by DNA inserts into the recombinant pSCREEN-T vector can be purified and used as immunogens as described below in Example 6.

The peptide sequences obtained from the screening of BVH-3 and BVH-11-2 gene libraries with the Mabs are in agreement with the Mab ELISA reactivities against the truncated gene products. As expected, the amino acid sequences obtained from H11-7G11 contained the sequence HGDHXH. These findings provide additional evidence for the location of epitopes recognized with the Mabs. Interestingly, although the Mabs H112-10G9, H112-10A2 and H11B-11B8 were reactive against the same peptide sequence (amino acid residues 594 to 679 on BVH-11-2 protein sequence), clones corresponding to the sequence spanning from amino acid residues 658 to 698 were only picked up by Mab H11B-11B8 thus revealing the location of H11B-11B8 epitope between amino acid residues 658 to 679 (SEQ ID NO: 163). Mabs H112-10G9, H112-10A2, and H11B-11B8 are directed against 3 distinct non overlapping epitopes located closely on the peptide sequence corresponding to amino acid residues 594 to 679 (SEQ ID NO: 22).

Table 5. Peptide sequences obtained from the screening of BVH-3 and BVH-11-2 gene libraries with Mabs

Mab	Clone/ Protein designation	Nucleotide position	Amino acid position	Amino acid sequence	SEQ ID NO
H3-4D4	4D4.9	SEQ ID 1: 226-509	SEQ ID 6: 76-169	DQGYVTSHGDHYHYYNGKVPYDALFSEELLMKDPNYQLKDA DIVNEVKGGYIIKVDGKYYVYLKDAAHADNVRTKDEINRQK QEHVKDNEKVNS	11
H11-7G11	7G11.7	SEQ ID 1: 193-316	SEQ ID 6: 64-105	GIQAEQIVIKITDQGYVTSHGDHYHYYNGKVPYDALFSEEL L	12
H11-7G11	7G11.9	SEQ ID 1: 1171-1284	SEQ ID 6: 390-428	TAYIVRHGDHFHYIPKSNQIGQPTLPNNSLATPSPSLPI	13
H3-4D3	4D3.4	SEQ ID 1: 2565-2670	SEQ ID 6: 855-890	TSNSTLEEVPTVDPVQEKVAKFAESYGMKLENVLFN	14
HN1-8E3	8E3.1	SEQ ID 1: 3004-3120	SEQ ID 6: 1016-1039	MDGTIELRLPSGEVIKKNLSDFIA	15
HN1-1G2	1G2.2	SEQ ID 1: 3017-3120	SEQ ID 6: 1005-1039	YGLGLDSVIFNMDGTIELRLPSGEVIKKNLSDFIA	16
HN1-10C12	10C12.7	SEQ ID 1: 2936-3120	SEQ ID 6: 983-1039	PALEEAPAVDPVQEKLEKFTASYGLGLDSVIFNMDGTIELR LPSGEVIKKNLSDFIA	17
HN1-14F6	14F6.3	SEQ ID 1: 2501-2618	SEQ ID 6: 833-872	KVEEPKTSEKVEKEKLSETGNSTSNSTLEEVPTVDPVQEK	18

Mab	Clone/ Protein designation	Nucleotide position	Amino acid position	Amino acid sequence	SEQ ID NO
HN1-12D8	B12D8.2	SEQ ID 1:1433-1767	SEQ ID 6: 512-589	MKDLDKKIEEKIAGIMKQYGVKRESIVVNKEKNAIIYPHGD HHHADPIDEHKPVGIGHSHSNYELFKPEEGVAKKEGN	19
H3V-7F4	7F4.1	SEQ ID 1: 1633-1785	SEQ ID 6: 545-595	AIIYPHGDHHHADPIDEHKPVGIGHSHSNYELFKPEEGVAK KEGNKVYTGE	20
H112-10D7	10D7.5	SEQ ID 5: 1685-1765	SEQ ID 8: 525-553	IQVAKLAGKYTTEDGYIFDPRDITSDEGD	21
H112-10G9	10G9.3	SEQ ID 5: 1893-2150	SEQ ID 8: 594-679	DHQDSGNTEAKGAEAIYNRVKAAKKVPLDRMPYNLQYTVEV KNGSLIIPHYDHYHNIKFEWFDEGLYEAPKGYSLEDLLATV KYYV	22
H112-10A2	10A2.2	SEQ ID 5: 1893-2150	SEQ ID 8: 594-679	DHQDSGNTEAKGAEAIYNRVKAAKKVPLDRMPYNLQYTVEV KNGSLIIPHYDHYHNIKFEWFDEGLYEAPKGYSLEDLLATV KYYV	22
H11B-11B8	B11B8.1	SEQ ID 5: 1893-2150	SEQ ID 8: 594-679	DHQDSGNTEAKGAEAIYNRVKAAKKVPLDRMPYNLQYTVEV KNGSLIIPHYDHYHNIKFEWFDEGLYEAPKGYSLEDLLATV KYYV	22
H11B-11B8	11B8.4	SEQ ID 5: 2085-2217	SEQ ID 8: 658-698	GLYEAPKGYSLEDLLATVKYYVEHPNERPHSDNGFGNASDH	23

Mab	Clone/ Protein designation	Nucleotide position	Amino acid position	Amino acid sequence	SEQ ID NO
H112-3A4	3A4.1	SEQ ID 5: 2421-2626	SEQ ID 8: 769-837	VENSVINAKIADAEALLEKVTDPSIRQNAMETLTGLKSSLL LGTKDNNTISAEVDSLLALLKESQPAPI	24

EXAMPLE 4

This example describes the immunization of animals with recombinant proteins for the generation of antibody reactive with BVH-3, BVH-11 and/or BVH-11-2.

NZW rabbits (Charles River Laboratories, St-Constant, Québec, Canada) were immunized subcutaneously at multiple sites with 50 μg or 100 μg of the purified BVH-3M, L-BVH-3AD, NEW1, NEW13, or L-BVH-11 recombinant protein in presence of 80 μg of QuilA adjuvant (Cedarlane Laboratoratories Ltd, Hornby, Canada). The rabbits were boosted two times at three-week intervals with the same antigen and blood samples were collected before each immunization and 6 to 28 days following the last immunization. The sera samples were designated preimmune, post 1st, post 2nd or post 3rd injection. The rabbit immune response to immunization was evaluated by ELISA using recombinant BVH-3M (BVH-3M-His•Tag fusion protein/ pET21 system) or BVH-11M (BVH-11M-His•Tag fusion protein/ pET21 system) proteins or suspensions of heat-killed S. pneumoniae Rx-1 cells as coating antigens. ELISA titer was defined as the reciprocal of the highest sera dilution at which absorbance A_{410} value was 0.1 above the background value. Antibodies reactive with BVH-3 and/or BVH-11 epitopes were elicited following immunization in all animals as shown in the following Table 6. Antibody reactive with recombinant or pneumococcal antigens was not present in the preimmune sera. The immune response to immunization was detectable in the sera of each rabbit after a single injection of recombinant antigen. The antibody response following the second injection with either antigen tested was characterized by a strong increase in antibody titer. Interestingly, good titers of antibody reactive with S. pneumoniae cells, with an average titer of 52,000 after the third immunization, were obtained, thus establishing that native pneumococcal epitopes are expressed on the recombinant E. coli gene products. These

data support the potential use of BVH-3, BVH-11 and/or BVH-11-2 gene products and the antibody raised to BVH-3, BVH-11 and/or BVH-11-2 gene products as vaccines for the prevention and the treatment of pneumococcal disease, respectively.

Table 6. Rabbit Antibody response to immunization with BVH-3 and BVH-11 gene products

			ELISA Titer with coating antigen		
Rabbit	Immunogen	Sera sample	BVH-3M	BVH-11M	S. pneumoniae
#15	BVH-3M (50μg)	Preimmune	NT	NT	200
		Post-1st	NT	NT	1,600
		Post-2nd	NT	NT	20,000
		Post-3rd	512,000	NT	40,000
#16	BVH-3M (100μg)	Preimmune	NT	NT	200
		post 1st	NT	NT	1,600
		post 2nd	NT	NT	40,000
		post 3rd	10^6	NT	80,000
#112	L-BVH-3AD (50 μg)	Preimmune	<100	NT	NT
		post 1st	16,000	NT	NT
		post 2nd	512,000	NT	NT
		post 3rd	$2x10^6$	NT	32,000
#113	New 1 (50 μg)	Preimmune	<100	NT	NT
		post 1st	16,000	NT	NT
		post 2nd	512,000	NT	NT
		post 3rd	10^6	NT	64,000
#114	New 13 (50 μg)	Preimmune	NT	<100	NT
		post 1st	NT	16,000	NT
		post 2nd	NT	64,000	NT
		post 3rd	NT	256,000	32,000
		Preimmune	NT	<100	NT

#116	L-BVH-11 (50 μg)	post 1st.	NT	64,000	NT
		post 2nd	NT	10^6	NT
		post 3rd	NT	$2x10^6$	64,000

NT : not tested

EXAMPLE 5

This example describes the protection of animals against fatal experimental pneumococcal infection by administration of antibody raised to BVH-3, BVH-11 or BVH-11-2 gene products.

High-titer Mab preparations were obtained from ascites fluid of mice inoculated intraperitoneally with Mab-secreting hybridoma cells according to the method described by Brodeur et al (J Immunol Methods 71 :265-272, 1984). Sera samples were collected from rabbits immunized with BVH-3M as described in Example 4. The rabbit sera collected after the third immunization and ascites fluid were used for the purification of antibodies by precipitation using 45 to 50% saturated ammonium sulfate. The antibody preparations were dissolved and dialyzed against phosphate-buffered saline (PBS).

CBA/N (xid) mice (National Cancer Institute, Frederick, MA) were injected intraperitoneally with either 0.1 ml of purified rabbit antibodies or 0.2 ml of ascites fluid before intravenous challenge with approximately 200 CFU of the type 3 S. pneumoniae strain WU2. Control mice received sterile PBS or antibodies purified from preimmune rabbit sera or sera from rabbits immunized with an unrelated N. meningitidis recombinant protein antigen. One group of mice was challenged with S. pneumoniae before the administration of anti-BVH-3 antibody. Samples of the S. pneumoniae challenge inoculum were plated on chocolate agar plates to determine the number of CFU and verify the challenge dose. The CBA/N mice were chosen because of their high susceptibility to S. pneumoniae

infection. The LD_{50} of WU2 injected intravenously to CBA/N mice is estimated to be ≤10 CFU. Deaths were recorded at 24-h intervals for a period of at least 7 days.

The protection data obtained from mice injected with rabbit anti-BVH-3 antibody are set forth in the following Table 7. Nine out of 10 mice receiving the anti-BVH-3 antibody survived the challenge in contrast to none of 10 mice injected with control antibody or PBS buffer. The observation that antibody raised to the BVH-3-M molecule passively protected even when administered after the challenge demonstrated the ability of anti-BVH-3 antibody to prevent death even from an already established infection.

Table 7. Protective effects of rabbit antibody to BVH-3-M gene in CBA/N mice challenged i.v. with WU2 pneumococci

Antibody preparation	Time of antibody administration	Alive : Dead	Days to death post-infection
Anti-BVH3M	1 h before infection	5 : 0	>14, >14, >14, >14, >14
Anti-N. meningitidis	1 h before infection	0 : 5	2, 2, 2, 2, 2
Anti-BVH-3M	0.5 h post-infection	4 : 1	2, >14, >14, >14, >14
None (PBS)	1 h before infection	0 : 5	1, 2, 2, 2, 2

CBA/N mice were infected with 1000 CFU of WU2 S. pneumoniae before or after intraperitoneal administration of 0.1 ml of rabbit antibody.

In an other experiment, 0.1 ml of rabbit antibody prepared from preimmune and immune sera were administered intraperitoneally to CBA/N mice four hours before intranasal challenge with 280 CFU of S. pneumoniae P4241 type 3 strain. As seen in the following Table 8, all immunized mice survived

the challenge while none of 9 mice receiving preimmune sera antibody or buffer alone were alive on day 6 post-infection. S. pneumoniae hemocultures on day 11 post-challenge were negative for all surviving mice. Furthermore, 100% protection was observed in mice receiving monoclonal antibodies H112-10G9 or a mixture of H112-10G9 and H11B-7E11 which are directed against BVH-11/BVH-11-2.

Table 8. Protective effects of passive transfer of rabbit antibody to BVH-3-M gene product or anti-BVH-11/BVH-11-2 specific Mabs in CBA/N mice challenged i.n. with P4241 pneumococci

Antibody preparation	Alive : Dead	Days to death post-infection
Anti-BVH-3M	5 : 0	>11, >11, >11, >11, >11
Antibody from preimmune sera	0 : 5	3, 3, 3, 6, 6
H112-10G9	4 : 0	>11, >11, >11, >11
H112-10G9+H11B-7E11	5 : 0	>11, >11, >11, >11, >11
None (PBS)	0 : 4	3, 3, 3, 3

Altogether, the results from Table 7 and Table 8 clearly establish that immunization of animals with a BVH-3 gene product such as BVH-3M elicited protective antibodies capable of preventing experimental bacteremia and pneumonia infections.

The protection data obtained for mice injected with ascites fluid are set forth in the following Table 9. Administration of a volume of 0.2 ml of ascites fluid of 0.2 ml of some sets of ascites fluid prevented death from experimental infection. For example, H112-3A4 + H112-10G9 and H112-10G2 + H112-10D7

sets of 2 Mabs conferred complete protection against experimental infection. These data indicated that antibody targetting BVH-11 and/or BVH-11-2 epitopes gave efficient protection. The Mabs H112-3A4, H112-10G9, H112-10D7, H112-10A2, H112-3E8, H112-10C5, H11B-11B8, H11B-15G2, H11B-1C9, H11B-7E11, H11B-13D5 and H11-10B8 were present in at least one protective pair of Mabs and were said to be protective and reactive against protective epitopes. The locations of protection-conferring epitopes on BVH-11-2 molecules are summarized in Table 10 and Figure 29 . Protective Mabs H112-3A4, H112-10G9, H112-10D7, H112-10A2, H112-3E8, H112-10C5, H11B-11B8, H11B-15G2, H11B-1C9, H11B-7E11, H11B-13D5 and H11-10B8 were all reactive with New 10 protein corresponding to amino acid residues 271 to 838 on the BVH-11-2 molecule. Six out of these 12 Mabs were directed against epitopes present in the NEW 19 protein and 3 protective Mabs recognized NEW 14. Interestingly, Mab H112-3A4 and H112-10C5 reacted with distinct epitopes exclusive to BVH-11-2 located at the carboxyl end comprised between amino acid residues 769 and 837. Also, Mabs H11-7G11, H11-6E7 and H3-4F9 reactive with epitopes shared by pneumococcal BVH-3, BVH-11 and BVH-11-2 molecules did not succeed to protect even if given in combination with protective H112-10G9 or H112-11B8 Mab. These Mabs recognized epitopes located at the amino end of the BVH-3, BVH-11 and BVH-11-2 molecules comprising, respectively, the first 225, 228 and 226 amino acid residues. The comparison of the BVH-3, BVH-11 and BVH-11-2 protein sequences revealed that a large number of amino acids were conserved in the amino end portion comprising these 225-228 residues with a global 72.8 % identity (Figure 32).

Altogether the data set forth in Table 9 and Table 10 suggest that the protection eliciting BVH-11- and BVH-11-2-epitopes is comprised in the carboxy terminal product containing amino acids 229 to 840 and 227 to 838, on BVH-11 and BVH-11-2 proteins, respectively.

Table 9. Passive immunization with BVH-11- and/or BVH-11-2-specific Mabs can protect mice from lethal experimental pneumococcal infection.

Experiment	Mab	Alive : Dead	Days to death post-infection
1	H112 3A4 + H112-10G9	6 : 0	6 X >10
	H112-3A4 + H112-10D7	5 : 1	4, 5X >10
	None	0 : 6	2, 2, 2, 2, 2, 6
2	H112-10 A2 + H112-10D7	5 : 1	3, 5X >10
	H112-3E8 + H112-10G9	6 : 0	6 X >10
	None	0 : 6	2, 2, 2, 2, 2, 2
3	H112-10D7 + H11B-11B8	6 : 0	6 X >10
	H112-10G9 + H11B-15G2	3 : 3	2, 6, 6, 3 X >10
	None	0 : 6	2, 2, 2, 2, 2, 2
4	H112-10G9 + H112-10D7	5 : 0	5 X >11
	None	0 : 5	2, 2, 2, 2, 2
5	H112-10G9 + H11-10B8	4 : 1	8, 4 X >14
	H112-10G9 + H11B-7E11	5 : 0	5 X >14
	None	0 : 3	1, 2, 2
6	H112-10G9 + H11B-1C9	4 : 1	4, 4 X >14
	None	0 : 3	2, 2, 2
7	H112-10C5 + H11B-13D5	5 :0	5 X >14
	None	3 :3	2,2,2

CBA/N mice were injected intraperitoneally with a total of 0.2 ml of ascites fluid 4 hours before intravenous challenge with S. pneumoniae WU2.

Table 10. Deduced locations of protection-conferring epitopes on BVH-11-2 molecules.

Mabs	Protection	Gene products carrying Mab-epitope
H112-3A4	+	NEW 19 and NEW 11
H112-10G9	+	NEW 19
H112-10D7	+	NEW 14 and NEW 10
H112-10A2	+	NEW 19
H112-3E8	+	NEW 19
H11B-11B8	+	NEW 19
H11B-15G2	+	NEW 18
H11B-7E11	+	NEW 14 and NEW 10
H11-10B8	+	NEW 18
H11B-1C9	+	NEW 14 and NEW 10
H112-3A1	-	NEW 18 and NEW 8
H112-10H10	-	NEW 18 and NEW 8
H112-2H7	-	BVH-11-2M
H112-6H7	-	BVH-11-2M
H11-7G11	-	BVH-11A and BVH-3C
H11-6E7	-	BVH-11A and BVH-3C
H112-10C5	+	NEW 19, NEW11 and 3A4.1
H11B-13D5	+	NEW 19
H112-7G2	-	NEW 18
H112-7E8	-	BVH-11-2M
H3-4F9	-	BVH-11A and BVH-3C

Altogether the data presented in this example substantiate the potential use of antibodies raised to BVH-3, BVH-11 or BVH-11-2 molecules as therapeutic means to prevent, diagnose or treat S. pneumoniae diseases.

EXAMPLE 6

This example describes the localization of surface-exposed peptide domains using Mabs described in Example 1.

S. pneumoniae type 3 strain WU2 was grown in Todd Hewitt (TH) broth (Difco Laboratories, Detroit MI) enriched with 0.5% Yeast extract (Difco Laboratories) at 37°C in a 8% CO_2 atmosphere to give an OD_{600} of 0.260 (~10^8 CFU/ml). The bacterial suspension was then aliquoted in 1 ml samples and the S. pneumoniae cells were pelletted by centrifugation and resuspended in hybridoma culture supernatants. The bacterial suspensions were then incubated for 2 h at 4°C. Samples were washed twice in blocking buffer [PBS containing 2% bovine serum albumin (BSA)], and then 1 ml of goat fluorescein (FITC)-conjugated anti-mouse IgG + IgM diluted in blocking buffer was added. After an additional incubation of 60 min at room temperature, samples were washed twice in blocking buffer and fixed with 0.25 % formaldehyde in PBS buffer for 18-24 h at 4°C. Cells were washed once in PBS buffer and resuspended in 500 µl of PBS buffer. Cells were kept in the dark at 4°C until analyzed by flow cytometry (Epics® XL; Beckman Coulter, Inc.). Ten thousands (10,000) cells were analyzed per sample and the results were expressed as % Fluorescence and Fluorescence index (FI) values. The % Fluorescence is the number of fluorescein-labelled S. pneumoniae cells divided by 100 and the FI value is the median fluorescence value of pneumococci treated with Mab supernatant divided by the fluorescence value of pneumococci treated with the conjugate alone or with a control unrelated Mab. A FI value of 1 indicated that the Mab has not been detected at the surface of the bacteria whereas a FI value higher than 2 was considered positive when at least 10 % of the pneumococcal cells were labelled and indicated that the Mab was reactive with cell-surface exposed epitopes. The following Table 11 summarized the data obtained with the Mabs tested by flow cytometry.

Flow cytometric analysis revealed that the Mabs reactive with BVH-3C and/or BVH-11A molecules did not bind to the cell surface. In contrast, with the exception of Mabs H3V-9C6 and H3V-16A7, the Mabs reactive with NEW 1, NEW 2, NEW 3, NEW 22 or NEW 23 BVH-3 gene products were detected at the surface of pneumococci. These data indicated that the first 225 amino acid residues located at the amino end of BVH-3 are internal. The lack of binding of Mabs H3V-9C6 and H3V-16A7 suggest some portions of the sequence corresponding to the 177-amino acids absent from the BVH-3 molecule of S. pneumoniae SP63 appears not to be accessible to antibodies.

Results from BVH-11- and/or BVH-11-2-reactive Mabs revealed that there is a good correlation between surface-exposure and protection. All Mabs reactive with internal epitopes as determined by the flow cytometry assay were not protective whereas all the protective Mabs described in Example 5 gave a positive signal in flow cytometry. Although an FI value of 9.0 and a % Fluorescence of 81.2 were obtained with Mab H11-7G11, this Mab was not shown to protect. Additional assays can be used to further evaluate whether this Mab and its corresponding epitope might participate in anti-infectious immunity.

Table 11. Results from the binding of Mabs at the surface of S. pneumoniae by flow cytometry analysis

Mab	% Fluorescence	FI	Binding	Gene products carrying Mab-epitope
H3-4F9	3.4	1.2	-	BVH-3C and BVH-11A
H3-4D4	3.4	1.2	-	BVH-3C and BVH-11A
H3-9H12	2.5	1.1	-	BVH-3C and BVH-11A
H3-7G2	66.2	6.3	+	NEW 22
H3-10A1	58.8	5.6	+	NEW 23

Mab	% Fluorescence	FI	Binding	Gene products carrying Mab-epitope
H3-4D3	33.2	3.5	+	NEW 3
H3V-4F3	24.4	2.9	+	NEW 1
H3V-2F2	15.6	2.4	+	NEW 2
H3V-7F4	58.7	5.6	+	NEW 2
H3V-7H3	68.8	6.9	+	NEW 3
H3V-13B8	75.0	7.7	+	NEW 3
H3V-9C2	66.4	6.2	+	NEW 22
H3V-9C6	2.9	1.0	-	NEW 22
H3V-16A7	6.6	1.7	-	NEW 23
H3V-15A10	58.7	5.7	+	NEW 22 and NEW 23
HN1-5H3	43.4	5.3	+	NEW 1
HN1-8E3	57.4	6.6	+	NEW 1
HN1-14F6	57.8	6.7	+	NEW 1
HN1-2G2	54.8	6.3	+	NEW 2
HN1-12D8	14.3	3.0	+	NEW 2
HN1-14B2	11.5	2.7	+	NEW 2
HN1-1G2	59.9	7.0	+	NEW 3
HN1-10C12	13.6	2.8	+	NEW 3
H11-6E7	4.9	1.2	-	BVH-3C and BVH-11A
H11-10H10	6.5	1.6	-	BVH-3C and BVH-11A
H11-7G11	81.2	9.0	+	BVH-3C and NEW 2
H11-1B12	3.1	1.2	-	BVH-11A
H11-7B9	2.4	1.1	-	BVH-11A
H11-10B8	81.1	9.1	+	NEW 18 and NEW 8
H11-1A2	84.4	10	+	NEW 18 and NEW 8
H11-3H5	84.0	9.8	+	NEW 18 and NEW 8
H112-13C11	49.3	5.9	+	NEW 18 and NEW 8
H112-	0.4	1.0	-	BVH-11A and NEW 18

Mab	% Fluorescence	FI	Binding	Gene products carrying Mab-epitope
10H10				
H112-1D8	0.4	1.0	-	BVH-11A and NEW 18
H112-10G9	78.9	10.4	+	NEW 19
H112-10A2	75.5	9.6	+	NEW 19
H112-3E8	62.5	7.5	+	NEW 19
H112-10D7	64.5	7.7	+	NEW 14
H112-2H7	0.7	1.1	-	BVH-11A
H112-6H7	0.3	1.0	-	BVH-11A
H112-3A4	70.1	8.9	+	NEW 11
H112-10C5	86.3	9.2	+	NEW 11 AND 3A4.1
H112-14H6	89.6	11	+	NEW 11
H112-14H6	0.8	1.4	-	NEW 11
H112-7G2	4.7	2.0	-	NEW 18
H112-13H10	0.5	1.0	-	NEW 18
H112-7E8	0.4	1.0	-	BVH-11-2M
H112-7H6	0.2	1.0	-	BVH-11-2M
H11B-5F10	3.1	1.1	-	NEW 18
H11B-15G2	60.2	5.7	+	NEW 18 and NEW 8
H11B-13D5	75.7	8.3	+	NEW 19
H11B-11B8	78.4	8.3	+	NEW 19
H11B-	32.3	3.5	+	NEW 14

Mab	% Fluorescence	FI	Binding	Gene products carrying Mab-epitope
7E11				
H11B-1C9	57.3	5.5	+	NEW 14
H11B-5E3	1.8	1.0	-	NEW 7
H11B-6E8	2.4	1.0	-	NEW 7

EXAMPLE 7

This example describes the immunization of animals with peptide epitopes of BVH-3 and BVH-11-2.

The recombinant pSCREEN-T vector (Novagen, Madison, WI) containing DNA fragment (nucleotides 2421 to 2626 on SEQ ID NO : 5) encoding the Mab 3A4-epitope (SEQ ID NO: 24) was transformed by electroporation (Gene Pulser II apparatus, BIO-RAD Labs, Mississauga, Canada) into E. coli Tuner (λDE3) pLysS [BL21 (F' ompT hsdSB (rB^-mB^-) gal dcm lacYI pLysS (Cm^r)] (Novagen). In this strain, the expression of the fusion protein is controlled by the T7 promoter which is recognized by the T7 RNA polymerase (present on the λDE3 prophage, itself under the control of the lac promoter inducible by isopropyl-β-D-thiogalactopyranoside (IPTG). The pLysS plasmid reduces the basal fusion protein expression level by coding for a T7 lysozyme, which is a natural inhibitor of the T7 RNA polymerase.

The transformants were grown at 37°C with 250 RPM agitation in LB broth (peptone 10g/l, yeast extract 5g/l, NaCl 5g/l) supplemented with 50mM glucose, 100μg/ml carbenicillin and 34μg/ml chloramphenicol, until the absorbance at 600nm reached a value of 0,7. The overexpression of T7gene 10 protein-His•Tag-3A4.1 fusion protein was then induced by the addition of IPTG to a final concentration of 1mM and further

incubation at 25°C with 250 RPM agitation for 3 hours. Induced cells from a 800-ml culture were pelleted by centrifugation and frozen at -70°C. The fusion protein was purified from the soluble cell fraction by affinity chromatography based on the binding of a six histidine residues sequence (His-Tag) to divalent cations (Ni^{2+}) immobilized on a metal chelation Ni-NTA resin (Qiagen, Mississauga, Canada). Briefly, the pelleted cells were thawed and resuspended in Tris buffered sucrose solution (50mM Tris, 25%(w/v) sucrose) and frozen at -70°C for 15 minutes. Cells were incubated 15 minutes on ice in the presence of 2mg/ml lysozyme before disruption by sonication. The lysate was centrifuged at 12000 RPM for 30 minutes and Nickel charged Ni-NTA resin (QIAgen) was added to the supernatant for an overnight incubation at 4°C, with 100 RPM agitation. After washing the resin with a buffer consisting of 20mM Tris, 500mM NaCl, 20mM imidazole pH 7,9, the fusion 3A4.1 protein was eluted with the same buffer supplemented with 250mM imidazole. The removal of the salt and imidazole was done by dialysis against PBS at 4°C. The protein concentration was determined with BCA protein assay reagent kit (Perce, Rockford,IL) and adjusted to 760 µg/ml.

To evaluate whether immunization with an epitope peptide sequence could confer protection against disease, groups of 6 female CBA/N (xid) mice (National Cancer Institute) are immunized subcutaneously three times at three-week intervals with affinity purified T7gene10 protein-His•Tag-3A4.1 fusion protein or, as control, with QuilA adjuvant alone in PBS. Twelve to fourteen days following the third immunization, the mice are challenged intravenously with S. pneumoniae WU2 strain or intranasally with P4241 strain. Samples of the S. pneumoniae challenge inoculum are plated on chocolate agar plates to determine the number of CFU and to verify the challenge dose. The challenge dose are approximalety 300 CFU. Deaths are recorded daily for a period of 14 days and on day

14 post-challenge, the surviving mice are sacrificed and blood samples tested for the presence of S. pneumoniae organisms. The 3A4.1 protein or other tested protein is said protective when the number of mice surviving the infection or the median number of days to death is significantly greater in the 3A4.1-immunized group compared to the control mock-immunized group.

EXAMPLE 8

This example illustrates the improvement of the antibody response to pneumococci using BVH-3 fragments and variants thereof.

The combined results obtained from studies of Mab reactivity with truncated gene products, epitope-expressing colonies and live intact pneumococci presented in examples 2, 3 and 6, allowed to delineate between surface-exposed and internal epitopes. The epitopes detected by Mabs that efficiently bound to pneumococci cells mapped to a region comprised between amino acid residues 223 to 1039 of BVH-3 described in SEQ ID NO 6. The existence of protective epitopes in the BVH-3-carboxyl half was confirmed by demonstrating that mice immunized with NEW1 molecule were protected from fatal infection with P4241 strain.

Gene sequence comparison revealed that in some strains, the region of BVH-3 encoding for amino acids 244 to 420 as described in SEQ ID NO6 is absent thus suggesting the lack of utility of this sequence in vaccine to prevent disease caused by such strains (SEQ ID NO: 9 versus SEQ ID NO: 1). Further BVH-3 fragments or variants thereof were designed in the purpose to develop a universal highly effective vaccine that would target the immune response to ubiquitous surface-exposed protective epitopes. BVH-3 gene fragments designated NEW1 (encoding amino acid residues 472 to 1039 from SEQ ID NO: 6) and NEW40 (encoding amino acid residues 408 to 1039 from SEQ

ID NO: 6) were amplified from the S. pneumoniae strain SP64 by PCR using pairs of oligonucleotides engineered for the amplification of the appropriate gene fragment. Each of the primers had a restriction endonuclease site at the 5'end, thereby allowing directional in-frame cloning of the amplified product into the digested plasmid vector. PCR-amplified products were digested with restriction endonucleases and ligated to linearized plasmid pET21 (Novagen) expression vector digested likewise. Oligonucleotide primers HAMJ489 (ccgaattccatatgcaaattgggcaaccgactc; NdeI) and HAMJ279 (cgccaagcttcgctatgaaatcagataaattc; HindIII) were used for the NEW 40 construction. Clones were first stabilized in E. coli DH5α before introduction into E. coli BL21 (λDE3) for expression of the truncated gene products. Variants from NEW1 and NEW40 were generated by mutagenesis using the Quickchange Site-Directed Mutagenesis kit from Stratagene and the oligonucleotides designed to incorporate the appropriate mutation. The presence of 6 histidine tag residues on the C-terminus of the recombinant molecules simplified the purification of the proteins by nickel chromatography. The following tables 12 and 13 describe the sequences of the primers used for the mutagenesis experiments and the variant gene products generated, respectively. Mutagenesis experiments using primer sets 39, 40, 46, 47 or 48 resulted in silent changes and were performed in the purpose of improving the expression of the desired gene or gene fragment since it was observed that during the course of expression, BVH-3 gene and fragments of, shorter secondary translation initiation products were coexpressed.

Table 12. List of PCR oligonucleotide primer sets used for site-directed mutagenesis on BVH-3 gene truncates

Primer set	Primer identification	SEQ ID No	Primer SEQUENCE 5' ---> 3'
9	HAMJ513	177	GAATCAGGTTTTGTCATGAGTTCCGGAGACCACAATCATTATTTC
	HAMJ514	178	GAAATAATGATTGTGGTCTCCGGAACTCATGACAAAACCTGATTC
10	HAMJ515	179	GTCATGAGTTCCGGAGACTCCAATCATTATTTCTTCAAGAAGG
	HAMJ516	180	CCTTCTTGAAGAAATAATGATTGGAGTCTCCGGAACTCATGAC
11	HAMJ517	181	ATGAGTTCGGAGACTCCAATTCTTATTTCTTCAAGAAGGACTTG
	HAMJ518	182	CAAGTCCTTCTTGAAGAAATAAGAATTGGAGTCTCCGGAACTCAT
14	CHAN51	183	GCGATTATTTATCCGTCTGGAGATCACCATCATGC
	CHAN52	184	GCATGATGGTGATCTCCAGACGGATAAATAATCGC
17	CHAN53	185	CCGTCTGGAGATGGCCATCATGCAGATCCG
	CHAN54	186	CGGATCTGCATGATGGCCATCTCCAGACGG
19	CHAN47	187	CCGCAGGGAGATAAGCGTCATGCAGATCCGATTG
	CHAN48	188	CAATCGGATCTGCATGACGCTTATCTCCCTGCGG
20	CHAN55	189	CCGTCTGGAGATGGCACTCATGCAGATCCGATTG
	CHAN56	190	CAATCGGATCTGCATGAGTGCCATCTCCAGACGG
22	CHAN57	191	CCGTCTGGAGATGGCACTTCTGCAGATCCGATTGATG
	CHAN58	192	CATCAATCGGATCTGCAGAAGTGCCATCTCCAGACGG
23	HAMJ523	193	CCGCATGGAGATGGCCATCATGCAGATCCG
	HAMJ524	194	CGGATCTGCATGATGGCCATCTCCATGCGG
24	HAMJ526	195	GTCATGAGTCACGGAGACTCCAATCATTATTTCTTCAAGAAGG
	HAMJ527	196	CCTTCTTGAAGAAATAATGATTGGAGTCTCCGTGACTCATGAC
25	HAMJ528	197	ATGAGTCACGGAGACCACAATTCTTATTTCTTCAAGAAGGACTTG
	HAMJ529	198	CAAGTCCTTCTTGAAGAAATAAGAATTGTGGTCTCCGTGACTCAT
29	HAMJ569	199	TACCTCATTATGACTCTTACTCTAACATCAAATTTGAGTGGTTTG
	HAMJ570	200	CAAACCACTCAAATTTGATGTTAGAGTAAGAGTCATAATGAGGTA
30	HAMJ571	201	TACCTTCTTATGACCATTACTCTAACATCAAATTTGAGTGGTTTG
	HAMJ572	202	AAACCACTCAAATTTGATGTTAGAGTAATGGTCATAAGAAGGTA
31	HAMJ573	203	AACGGTAGTTTAATCATACCTTCTAAAGACCATTACCATAACATC
	HAMJ574	204	GATGTTATGGTAATGGTCTTTAGAAGGTATGATTAAACTACCGTT

Primer set	Primer identification	SEQ ID No	Primer SEQUENCE 5' ---> 3'
32	HAMJ575	205	CGGTAGTTTAATCATACCTCATAAGGACTCTTACCATAACATCAAA
	HAMJ576	206	TTTGATGTTATGGTAAGAGTCCTTATGAGGTATGATTAAACTACCG
33	HAMJ577	207	AACGGTAGTTTAATCATACCTGACCATTACCATAACATCAAATTTG
	HAMJ578	208	CAAATTTGATGTTATGGTAATGGTCAGGTATGATTAAACTACCGTT
34	HAMJ579	209	AACGGTAGTTTAATCATACCTTACCATAACATCAAATTTGAGTGG
	HAMJ580	210	CCACTCAAATTTGATGTTATGGTAAGGTATGATTAAACTACCGTT
35	HAMJ581	211	ACCGGTAGTTTAATCATACCTAACATCAAATTTGAGTGGTTTGAC
	HAMJ582	212	GTCAAACCACTCAAATTTGATGTTAGGTATGATTAAACTACCGTT
37	HAMJ536	213	CCTATGTAACTCCACATATAACCCATAGCCACTGG
	HAMJ537	214	CCAGTGGCTATGGGTTATATGTGGAGTTACATAGG
39	HAMJ550	215	CGTGAAAGTATTGTCGTAAATAAAGAAAAAAATGCG
	HAMJ551	216	CGCATTTTTTTCTTTATTTACGACAATACTTTCACG
40	HAMJ586	217	CATGAAGAAGATGGTTACGGTTTCGATGCTAACCGTATTATCGCTGAAG
	HAMJ587	218	CTTCAGCGATAATACGGTTAGCATCGAAACCGTAACCATCTTCTTCTG
41	HAMJ588	219	GAATCAGGTTTTGTCATGAGTGACCACAATCATTATTTCTTC
	HAMJ589	220	GAAGAAATAATGATTGTGGTCACTCATGACAAAACCTGATTC
42	HAMJ590	221	GAAGATGAATCAGGTTTTGTCATGAGTAATCATTATTTCTTCAAG
	HAMJ591	222	CTTGAAGAAATAATGATTACTCATGACAAAACCTGATTCATCTTC
43	HAMJ592	223	GAAGATGAATCAGGTTTTGTCATGAGTTATTTCTTCAAGAAGGAC
	HAMJ593	224	GTCCTTCTTGAAGAAATAACTCATGACAAAACCTGATTCATCTTC
44	HAMJ594	225	AAAATGCGATTATTTATCCGCACCATCATGCAGATCCGATTG
	HAMJ595	226	CAATCGGATCTGCATGATGGTGCGGATAAATAATCGCATTTT
45	HAMJ600	227	AAAATGCGATTATTTATCCGGCAGATCCGATTGATGAACATAAAC
	HAMJ601	228	GTTTATGTTCCATCAATCGGATCTGCCGGATAAATAATCGCATTTT
46	HAMJ604	229	GATGCTAACCGTATAATCGCCGAAGACGAATCAGGTTTTGTCATG
	HAMJ605	230	CATGACAAAACCTGATTCGTCTTCGGCGATTATACGGTTAGCATC
47	HAMJ606	231	CGCCGAAGACGAATCCGGCTTTGTAATGAGTCACGGAGACTCC
	HAMJ607	232	GGAGTCTCCGTGACTCATTACAAAGCCGGATTCGTCTTCGGCG
48	HAMJ608	233	CATCTCATGAACAGGATTATCCCGGCAACGCCAAAGAAATGAAAG
	HAMJ609	234	CTTTCATTTCTTTGGCGTTGCCGGGATAATCCTGTTCATGAGATG

Table 13. Lists of truncated variant BVH-3 gene products generated from S. pneumoniae SP64

Protein designation	Gene/ Protein SEQ ID NO	Protein Identification*	PCR primer set (ref. table 12)	Gene used for mutagenesis
NEW1-mut1**	255	NEW1	39	NEW1
NEW35A	256	NEW1 550-SGDGTS-555	14,17,20,22	NEW1
NEW42	257	NEW40 55-SGDSNS-60 144-SGDGTS-149	9, 10, 11, 14, 17, 20, 22	NEW40
NEW49	258	NEW40 55-SGDHNH-60	9	NEW40
NEW50	259	NEW40 55-SGDSNH-60	10	NEW49
NEW51	260	NEW40 55-SGDHNH-60 144-SGDHHH-149	14	NEW49
NEW52	261	NEW40 55-SGDSNH-60 144-SGDGHH-149	10, 17	NEW51
NEW53	262	NEW40 55-HGDHNH-60 144-SGDHHH-149	14	NEW40
NEW54	263	NEW40 55-SGDHNH-60 144-SGDGHH-149	17	NEW53
NEW55	264	NEW1 550-HGDGHH-555	23	NEW1
NEW56	265	NEW40 55-HGDSNH-60 144-SGDHHH-149	24	NEW53
NEW56-mut2**	266	NEW56	40	NEW56
NEW56-mut3**	267	NEW56	46,47,48	NEW56
NEW57	268	NEW40 55-HGDHNS-60 144-SGDHHH-149	25	NEW53
NEW63	269	NEW40 55-HGDSNH-60 144-HGDHHH-149	24	NEW40
NEW64	270	NEW40 55-HGDHNS-60 144-HGDHHH-149	25	NEW40
NEW65	271	NEW40 55-HGDSNH-60 144-HGDGHH-149	23	NEW63

Protein designation	Gene/ Protein SEQ ID NO	Protein Identification*	PCR primer set (ref. table 12)	Gene used for mutagenesis
NEW66	272	NEW40 55-HGDHNS-60 144-HGDGHH-149	23	NEW64
NEW76	273	NEW40 55-HGDHNS-60 144-SGDGHH-149	17	NEW64
NEW105	274	NEW40 55-_____-60	41,42,43	NEW40
NEW106	275	New40 144-_____-149	44,45	NEW40
NEW107	276	NEW40 55-_____-60 144-_____-149	44,45	NEW105

* The underlined amino acid residues represent the modification in protein sequence. Nucleotides/amino acid residues are deleted in NEW105, NEW106 and NEW107 constructs.

** silent mutation, i.e. the polypeptide is the same as New1.

Groups of 7 or 8 female BALB/c mice (Charles River) immunized as described earlier in example 1 were used for protection experiments against intranasal challenge with virulent S. pneumoniae P4241 strain. The mice were observed for 10 to 14 days post-infection. Data from Table 15 clearly indicate that the NEW35A molecule was equivalent to the parental NEW1 in term of protection. Interestingly, high survival rates where obtained for NEW40- and NEW56-immunized groups with 7 and 8 survivors out of 8 animals, respectively. Similarly, NEW25 comprising amino acid residues 233 to 1039 protected 7 out of 8 animals from lethal infection.

Table 14. Protection mediated by BVH-3 fragments or variants thereof in experimental pneumonia

Experiment	Immunogen	Alive : Dead	Days to death post-infection
1	Quil A	0 : 8	4, 4, 4, 4, 4, 4, 4, 4
	NEW 1	5 : 3	5, 7, 7, >14, >14, >14, >14, >14
	NEW 35A	5 : 2	9, 10, >14, >14, >14, >14, >14
	NEW 40	7 : 1	13, >14, >14, >14, >14, >14, >14, >14
	BVH-3M	4 : 4	7, 8, 10, 12, >14, >14, >14, >14
2	Quil A	0 : 8	3, 3, 4, 4, 4, 4, 4, 4
	NEW 52	4 : 4	7, 7, 8, 9, >10, >10, >10, >10
	NEW56	8 : 0	8 X >10
	NEW 40	7 : 1	6, >10, >10, >10, >10, >10, >10, >10
3	QuilA	0 : 8	3, 3, 4, 4, 4, 4, 4, 4
	NEW25	7 : 1	6, >13, >13, >13, >13, >13, >13, >13

Additionally, flow cytometry analyses of the binding capacity of the sera antibodies from the vaccinated animals revealed that NEW40 and NEW56 antibodies labelled live intact pneumococci more efficiently than antibodies raised to BVH-3M (Table 15).

Table 15. Binding of mouse sera antibodies at the surface of S. pneumoniae type 3 strain WU2 as measured by flow cytometry.

Antisera	Fluorescence index			
	Experiment 1	Experiment 2	Experiment 3	Mean ± SE
BVH-3M	9.2	11.4	14.5	11.7 ± 1.5
NEW1	11.5	10.1	nd*	10.8 ± 0.7
NEW35A	14.3	12.9	nd	13.6 ± 0.7
NEW40	20.4	19.1	20.2	19.9 ± 0.4
NEW56	nd	16.7	20.2	18.5 ± 1.8
NEW52	nd	16.6	19.3	18.0 ± 1.4
Adjuvant alone	1.9	1.6	1.2	1.6 ± 0.2

* nd: not done

Cytometry results are expressed as fluorescence index value where the fluorescence index is the median fluorescence value of pneumococci treated with test sera divided by the background fluorescence value of pneumococci treated with the fluorescein conjugate alone. In these flow cytometric assays, all sera were used at a dilution of 1 :50 and the sera from mice immunized with BVH-3C fragment or QuilA adjuvant alone gave a value similar to the background value.

Altogether the protection and pneumococci antibody binding data indicate that vaccination using NEW1 or NEW40 molecules and variants thereof, directs the immune response to conserved protective surface-exposed epitopes.

EXAMPLE 9

This example describes the cloning and expression of a chimeric deletant BVH-11-2 gene encoding for a chimeric polypeptide corresponding to BVH-11-2 conserved protective surface-exposed epitopes present in most if not all S. pneumoniae strains.

BVH-11-2 gene fragments corresponding to 4 gene regions, were amplified by PCR using pairs of oligonucleotides engineered to amplify fragments originating from SEQ ID NO :5 spanning nucleotides 1662 to 1742, 1806 to 2153, 2193 to 2414 and 2484 to 2627 from S. pneumoniae strain Sp64 BVH-11-2 gene.

The primers used, HAMJ490-491, HAMJ492-HAMJ493, HAMJ494-HAMJ495, HAMJ496-HAMJ354 had a restriction endonuclease site at the 5' end, thereby allowing directional in-frame cloning of the amplified product into the digested pET21b(+) plasmid vector (Table 16). PCR-amplified products were digested with restriction endonucleases and ligated to linearized plasmid pSL301 vector digested likewise except for the PCR-amplified fragment obtained with the primer pair HAMJ490-HAMJ491. The HAMJ490-HAMJ491 PCR-amplified product was purified from agarose gel using a QIAquick gel extraction kit from QIAgen (Chatsworth, CA) and ligated into pGEM-T plasmid vector without any prior restriction endonuclease digestion. The resultant plasmid constructs were confirmed by nucleotide sequence analysis. The recombinant plasmids containing each of the four were digested with restriction endonucleases corresponding with the 5' end of each primer pair used for the PCR-amplification. The fragments were purified from agarose gel like described earlier and were all ligated to linearized plasmid pET21b (+) digested with the restriction enzymes NdeI and HindIII for the in-frame cloning of the four different regions of the BVH11-2 gene. Clones were first stabilized in E.coli DH5α before introduction into E.coli BL21 (λDE3) for expression of a chimeric pneumococcal protein molecule.

The resulting NEW43 gene sequence (SEQ ID No 257) is described in Figure 33.

The deduced amino acid sequence of NEW43 protein (SEQ ID No 258) is described in Figure 34.

Table 16. List of PCR oligonucleotide primers used to construct the NEW43 , VP43S and NEW86

Primer	SEQ ID NO	Sequence 5' - 3'	Nucleotide position	Restriction sites
HAMJ490	259	ccgaattccatatgcaaattacctacactgatgatg	SEQ ID 5 :1662-1683	NdeI
HAMJ491	260	ggactagtatcaaagataataaccgtcttc	SEQ ID 5 :1742-1722	SpeI
HAMJ492	261	ggactagttggattaaaaaagatagtttgtctg	SEQ ID 5 :1806-1830	SpeI
HAMJ493	262	ttcccgcggttcgacatagtacttgacagtcg	SEQ ID 5 :2153-2131	SacII
HAMJ494	263	ttcccgcggaacgctagtgaccatgttcg	SEQ ID 5 :2193-2212	SacII
HAMJ495	264	cggggtaccaggaatttcagcctcatctgtg	SEQ ID 5 :2414-2393	KpnI
HAMJ496	265	cccggtacccctagtattagacaaaatgctatggag	SEQ ID 5 :2484-2510	KpnI
HAMJ 354	65	cgccaagcttctgtataggagccggttgac	SEQ ID 5 :2627-2608	HindIII
HAMJ 583	266	ggatcccgggaggtatgattaaactaccg	SEQ ID 5 :2039-2021	SmaI
HAMJ 584	267	catgcccgggaacatcaaatttgagtggtttgac	SEQ ID 5 :2058-2081	SmaI
HAMJ 610	268	cttgatcgacatatgttggcaggcaagtacacaacag	SEQ ID 5 :1701-1722	NdeI

Table 17. List of truncated BVH-11-2 gene fragments generated from S. pneumoniae SP64 for the construction of NEW43

PCR-primer sets	Gene fragment designation	Corresponding amino acid residues on SEQ ID NO: 8	Cloning vector
HAMJ490-HAMJ491	NEW43a	517-543	pGEM-T
HAMJ492-HAMJ493	NEW43b	565-680	pSL301
HAMJ494-HAMJ495	NEW43c	694-767	pSL301
HAMJ496-HAMJ354	NEW43d	791-838	pSL301

Table 18. Properties of NEW86 and VP43S genes generated from NEW43 gene

PCR-primer sets	Gene/ Protein designation	Identification
HAMJ610-HAMJ354	VP43S	NEW43 C'end corresponding to residues 15-272)
HAMJ490-HAMJ583 HAMJ584-HAMJ354	NEW86	NEW43 109-__PG__-114

NEW43-derived molecules designated VP43S and NEW86 were generated from gene amplification and cloning experiments using PCR primers described in Tables 16 and 18 and pET21 expression plasmid vector. Variants from NEW43 were generated by mutagenesis using the Quickchange Site-Directed Mutagenesis kit from Stratagene and the oligonucleotides designed to incorporate the appropriate mutation. The presence of 6 histidine tag residues on the C-terminus of the recombinant molecules simplified the purification of the proteins by nickel chromatography. The following tables 19 and 20 describe the sequences of the primers used for the mutagenesis experiments and the NEW43 variant gene products generated, respectively.

Table 19. List of PCR oligonucleotide primer sets used for site-directed mutagenesis on NEW43 gene

Primer set	Primer identification	SEQ ID NO	Primer SEQUENCE 5′ ---> 3′
1	HAMJ 497	269	AACGGTAGTTTAATCATACCTTCTTATGACCATTACCATAACATC
	HAMJ 498	270	GATGTTATGGTAATGGTCATAAGAAGGTATGATTAAACTACCGTT
2	HAMJ499	271	AATCATACCTTCTTATGACTCTTACCATAACATCAAATTTGAGTG
	HAMJ500	272	CACTCAAATTTGATGTTATGGTAAGAGTCATAAGAAGGTATGATT
3	HAMJ501	273	TACCTTCTTATGACTCTTACTCTAACATCAAATTTGAGTGGTTTG
	HAMJ502	274	CAAACCACTCAAATTTGATGTTAGAGTAAGAGTCATAAGAAGGTA
26	HAMJ530	275	AATCATACCTCATTATGACTCTTACCATAACATCAAATTTGAGTG
	HAMJ531	276	CACTCAAATTTGATGTTATGGTAAGAGTCATAATGAGGTATGATT
27	HAMJ532	277	TACCTCATTATGACCATTACTCTAACATCAAATTTGAGTGGTTTG
	HAMJ533	278	CAAACCACTCAAATTTGATGTTAGAGTAATGGTCATAATGAGGTA
29	HAMJ569	279	TACCTCATTATGACTCTTACTCTAACATCAAATTTGAGTGGTTTG
	HAMJ570	280	CAAACCACTCAAATTTGATGTTAGAGTAAGAGTCATAATGAGGTA
30	HAMJ571	281	TACCTTCTTATGACCATTACTCTAACATCAAATTTGAGTGGTTTG
	HAMJ572	282	AAACCACTCAAATTTGATGTTAGAGTAATGGTCATAAGAAGGTA
31	HAMJ573	283	AACGGTAGTTTAATCATACCTTCTAAAGACCATTACCATAACATC
	HAMJ574	284	GATGTTATGGTAATGGTCTTTAGAAGGTATGATTAAACTACCGTT
32	HAMJ575	285	CGGTAGTTTAATCATACCTCATAAGGACTCTTACCATAACATCAAA
	HAMJ576	286	TTTGATGTTATGGTAAGAGTCCTTATGAGGTATGATTAAACTACCG
33	HAMJ577	287	AACGGTAGTTTAATCATACCTGACCATTACCATAACATCAAATTTG
	HAMJ578	288	CAAATTTGATGTTATGGTAATGGTCAGGTATGATTAAACTACCGTT
34	HAMJ579	289	AACGGTAGTTTAATCATACCTTACCATAACATCAAATTTGAGTGG
	HAMJ580	290	CCACTCAAATTTGATGTTATGGTAAGGTATGATTAAACTACCGTT
35	HAMJ581	291	ACCGGTAGTTTAATCATACCTAACATCAAATTTGAGTGGTTTGAC
	HAMJ582	292	GTCAAACCACTCAAATTTGATGTTAGGTATGATTAAACTACCGTT

Table 20. List of NEW43 variant gene products generated from S. pneumoniae SP64

Polypeptide designation	Polypeptide SEQ ID NO	Polypeptide identification*	PCR primer set (ref. table 22)	Gene used for mutagenesis
NEW60	293	NEW43 109-SYDHYH-114	1	NEW43
NEW61	294	NEW43 109-HYDSYH-114	26	NEW43
NEW62	295	NEW43 109-HYDHYS-114	27	NEW43
NEW80	296	NEW43 109-SYDSYH-114	2	NEW60
NEW81	297	NEW43 109-SYDSYS-114	3	NEW80
NEW82	298	NEW43 109-HYDSYS-114	29	NEW61
NEW83	299	NEW43 109-SYDHYS-114	30	NEW60
NEW84	300	NEW43 109-SKDHYH-114	31	NEW60
NEW85	301	NEW43 109-HKDSYH-114	32	NEW61
NEW88D1	302	NEW43 109-__DHYH-114	33	NEW43
NEW88D2	303	NEW43 109-____YH-114	34	NEW88D1
NEW88	304	NEW43 109-_____-114	35	NEW88D2

* The underlined amino acid residues represent the modification in protein sequence. Nucleotides/amino acid residues are deleted in NEW88D1, NEW88D2 and NEW88 constructs.

Groups of 7 or 8 female BALB/c mice (Charles River) immunized as described earlier in example 1 were used for protection experiments against intranasal challenge with virulent S. pneumoniae P4241 strain. Data from Table 21 clearly indicate that NEW 19, NEW43 and variants thereof provided protection against experimental pneumonia.

Table 21. Protection mediated by NEW19 and NEW43 fragments or variants thereof in experimental pneumonia

Experiment	Immunogen	Alive : Dead	Median day alive
1	Quil A	0 : 8	4, 4, 4, 4, 4, 4, 4, 5
	NEW 19	7 : 1	5, 7X >14
	NEW 43	8 : 0	8X >14
2	Quil A	0 : 8	4, 4, 4, 4, 4, 5, 5, 5
	NEW 43	7 : 1	8, 7X >14
	NEW 80	6 : 2	5, 6, 6 X >14
	NEW 83	6 : 2	8, 10, 6 X >14
3	Quil A	0 : 8	4, 4, 4, 4, 5, 5, 5, 5
	NEW 43	7 : 1	5, 7X >8
	NEW 88D1	5 : 3	5, 6, 6, 6 X >8
	NEW 88D2	5 : 3	6, 6, 6, 6 X >8
	NEW 88	7 : 1	6, 7X >8
3	Quil A	0 : 8	4, 4, 4, 5, 5, 5, 5, 6
	NEW 60	8 : 0	8 X >8
	NEW 84	8 : 0	8 X >8
	NEW 85	5 : 3	5, 7, 7, 5 X >8
	NEW 86	5 : 3	5, 6, 6, 5 X >8

EXAMPLE 10

This example describes the cloning and expression of chimeric genes encoding for a chimeric protein corresponding to the carboxy-terminal region of BVH-3 or variants thereof in fusion, at either the carboxyl end or the amino end, to NEW43 or variants thereof.

The chimeric genes comprising a BVH-3 truncate variant gene and a NEW43 or NEW43 variant gene have been designed following

the procedure described in example 1. The polypeptides encoded by these chimeric genes are listed in the table 22. Briefly, gene fragments to be included in a chimeric gene were amplified by PCR using pairs of oligonucleotides engineered so that the primers had a restriction endonuclease site at the 5' end, thereby allowing directional in-frame cloning of the amplified product into digested plasmid vectors (Table 23 and Table 24). PCR-amplified products were digested with restriction endonucleases and ligated to linearized plasmid pSL301 vector. The resultant plasmid construct were confirmed by nucleotide sequence analysis. The recombinant pSL301 plasmids containing a PCR product were redigested with the same endonuclease restriction enzyme for the obtention of the DNA inserts. The resulting insert DNA fragments were purified and inserts corresponding to a given chimeric gene were ligated into pURV22-NdeI vector for the generation of a chimeric gene. The expressed recombinant proteins were purified from supernatant fractions obtained from centrifugation of sonicated heat-induced E. coli cultures using multiple chromatographic purification steps.

Table 22. List of polypeptides encoded by chimeric genes comprising a BVH-3 truncate variant gene and a NEW43 or NEW43 variant gene

Polypeptide designation	SEQ ID NO	Identification
VP 89	327	M-New56 -GP- New43*
VP 90	328	M-New43 -GP- New56
VP 91	329	M-New52 -GP- New43
VP 92	330	M-New43 -GP- New52
VP 93	331	M-New56 -GP- New60
VP 94	332	M-New60 -GP- New56
VP 108	333	M-New56 -GP- New88
VP109	334	M-New88 -GP- New56

Polypeptide designation	SEQ ID NO	Identification
VP 110	335	M-New60 -GP- New105
VP 111	336	M-New60 -GP- New107
VP112	337	M-New88 -GP- New105
VP113	338	M-New88 -GP- New107
VP114	339	M-New80-GP- New105
VP115	340	M-New80 -GP- New107
VP116	341	M-New83 -GP- New105
VP117	342	M-New83 -GP- New107
VP119	343	M-New43S- GP-New105
VP120	344	M-New43S- GP-New107
VP121	345	M-New80S- GP-New105
VP122	346	M-New80S- GP-New107
VP123	347	M-New88S- GP-New105
VP124	348	M-New88S- GP-New107

* Encoded amino acids for the chimeras are expressed as the gene product, additional amino acid residues were added. M is methionine, G is glycine and P is proline.

Table 23. List of PCR oligonucleotide primer pairs designed for the generation of the chimeric genes encoding the polypeptides listed in Table 22.

Primer set	PCR-primer identification	Gene used for PCR amplification	Corresponding position of the gene fragment on
49	HAMJ490-HAMJ471	Variant New43	N-terminal
50	HAMJ564-HAMJ556	Variant New43	C-terminal
51	HAMJ489-HAMJ359	Variant New40	N-terminal
52	HAMJ559-HAMJ557	Variant New40	C-terminal
53	HAMJ610-HAMJ471	Variant New43S	N-terminal

Table 24. List of PCR oligonucleotide primers designed for the generation of the chimeric genes encoding the polypeptides listed in Table 22.

Primer	SEQ ID NO	Sequence 5' - 3'	Restriction site
HAMJ490	259	ccgaattccatatgcaaattaccta cactgatgatg	NdeI
HAMJ471	168	atatgggcccctgtataggagccgg ttgactttc	ApaI
HAMJ564	327	atatgggccccaaattacctacact gatgatgagattcagg	ApaI
HAMJ556	328	ataagaatgcggccgcctactgtat aggagccggttgactttc	NotI
HAMJ489	329	ccgaattccatatgcaaattgggca accgactc	NdeI
HAMJ359	173	tcccgggccccgctatgaaatcaga taaattc	ApaI
HAMJ559	330	atatgggccccaaattgggcaaccg actc	ApaI
HAMJ354	65	cgccaagcttctgtataggagccgg ttgac	HindIII
HAMJ610	268	cttgatcgacatatgttggcaggca agtacacaacag	NdeI
HAMJ557	331	ataagaatgcggccgcttacgctat gaaatcagataaattc	NotI
HAMJ279	35	cgccaagcttcgctatgaaatcaga taaattc	HindIII

What is claimed is:

1. An isolated polynucleotide comprising a polynucleotide chosen from;

(a) a polynucleotide encoding a polypeptide having at least 70% identity to a second polypeptide chosen from: table B, E or H;
(b) a polynucleotide encoding a polypeptide having at least 95% identity to a second polypeptide chosen from: table B, E or H;
(c) a polynucleotide encoding a polypeptide having an amino sequence chosen from table B, E or H or fragments, analogs or derivatives thereof;
(d) a polynucleotide encoding a polypeptide chosen from: table B, E or H;
(e) a polynucleotide encoding a polypeptide capable of generating antibodies having binding specificity for a polypeptide having a sequence chosen from: table B, E or H,
(f) a polynucleotide encoding an epitope bearing portion of a polypeptide chosen from table B, E or H; and
(g) a polynycleotide complementary to a polynucleotide in (a), (b), (c), (d),(e) or (f).

2. The isolated polynucleotide of claim 1 wherein said polynucleotide is (a).
3. The isolated polynucleotide of claim 1 wherein said polynucleotide is (b).
4. The isolated polynucleotide of claim 1 wherein said polynucleotide is (c).
5. The isolated polynucleotide of claim 1 wherein said polynucleotide is (d).
6. The isolated polynucleotide of claim 1 wherein said polynucleotide is (e).

7. The isolated polynucleotide of claim 1 wherein said polynucleotide is (f).
8. The isolated polynucleotide of claim 1 wherein said polynucleotide is (g).
9. The isolated polynucleotide of claim 7 wherein said polynucleotide is chosen from table B.

10. The isolated polynucleotide of claim 9 wherein said epitope bearing portion is chosen from table C.

11. The isolated polynucleotide of claim 7 wherein said polynucleotide is chosen from table E.

12. The isolated polynucleotide of claim 11 wherein said epitope bearing portion is chosen from table F.

13. The polynucleotide of anyone of claims 1 to 12, wherein said polynucleotide is DNA.

14. The polynucleotide of anyone of claims 1 to 12, wherein said polynucleotide is RNA.

15. A vector comprising the polynucleotide of claim 13, wherein said DNA is operably linked to an expression control region.

16. A host cell transfected with the vector of claim 15.

17. A process for producing a polypeptide comprising culturing a host cell according to claim 16 under conditions suitable for expression of said polypeptide.

18. An isolated polypeptide comprising a member chosen from:

(a) a polypeptide having at least 70% identity to a second polypeptide having an amino acid sequence chosen from: table B, E or H;

(b) a polypeptide having at least 95% identity to a second polypeptide having an amino acid sequence chosen from: table B, E or H;

(c) a polypeptide having an amino acid sequence chosen from table B, E or H;

(d) a polypeptide having amino acid sequence chosen from: table B, E or H or fragments, analogs or derivatives thereof;

(e) a polypeptide capable of generating antibodies having binding specificity for a second polypeptide having a sequence chosen from table B, E or H;

(f) an epitope bearing portion of a polypeptide having an amino acid sequence chosen from: table B, E or H;

(g) the polypeptide of (a), (b), (c), (d), (e), or (f) wherein wherein the N-terminal Met residue is deleted; or

(h) the polypeptide of (a), (b), (c), (d), (e), or (f) wherein the secretory amino acid sequence is deleted.

19. The polypeptide of claim 18 wherein said polypeptide is (f).

20. The polypeptide of claim 19 wherein said is chosen from table B.

21. The polypeptide of claim 20 wherein said epitope bearing portion is chosen from table C.

22. The polypeptide of claim 19 wherein said is chosen from table E.

23. The polypeptide of claim 22 wherein said epitope bearing portion is chosen from table F.

24. A chimeric polypeptide comprising two or more polypeptides chosen from table B, E or H thereof; provided that the polypeptides are linked as to form a chimeric polypeptide.

25. A vaccine composition comprising a polypeptide according to any one of claims 18 to 24 and a pharmaceutically acceptable carrier, diluent or adjuvant.

26. A method for therapeutic or prophylactic treatment of meningitis, otitis media, bacteremia or pneumonia infection in an individual susceptible to meningitis, otitis media, bacteremia or pneumonia infection comprising administering to said individual a therapeutic or prophylactic amount of a composition according to claim 25.

27. A method for therapeutic or prophylactic treatment of streptococcal bacterial infection in an individual susceptible to streptococcal infection comprising administering to said individual a therapeutic or prophylactic amount of a composition according to claim 25.

28. A method according to claim 26, wherein said individual is a mammal.

29. A method according to claim 27, wherein said individual is a mammal

30. A method according to claim 26, wherein said individual is a human.

31. A method according to claim 27, wherein said individual is a human

32. A method according to claim 27, wherein said bacterial infection is S.pneumoniae, group A *streptococcus*

(*pyogenes*), group B *streptococcus* (GBS or *agalactiae*), *dysgalactiae*, *uberis*, *nocardia* or *Staphylococcus aureus*.

33. A method according to claim 27, wherein said bacterial infection is S.pneumoniae.

34. Use of a vaccine composition according to claim 25 for the prophylactic or therapeutic treatment of Streptococcal infection in an animal susceptible to or infected with streptococcal infection comprising administering to said animal a prophylactic or therapeutic amount of the composition.

ATGAAATTTA GTAAAAAATA TATAGCAGCT GGATCAGCTG TTATCGTATC CTTGAGTCTA 60
TGTGCCTATG CACTAAACCA GCATCGTTCG CAGGAAAATA AGGACAATAA TCGTGTCTCT 120
TATGTGGATG GCAGCCAGTC AAGTCAGAAA AGTGAAAACT TGACACCAGA CCAGGTTAGC 180
CAGAAAGAAG GAATTCAGGC TGAGCAAATT GTAATCAAAA TTACAGATCA GGGCTATGTA 240
ACGTCACACG GTGACCACTA TCATTACTAT AATGGGAAAG TTCCTTATGA TGCCCTCTTT 300
AGTGAAGAAC TCTTGATGAA GGATCCAAAC TATCAACTTA AAGACGCTGA TATTGTCAAT 360
GAAGTCAAGG GTGGTTATAT CATCAAGGTC GATGGAAAAT ATTATGTCTA CCTGAAAGAT 420
GCAGCTCATG CTGATAATGT TCGAACTAAA GATGAAATCA ATCGTCAAAA ACAAGAACAT 480
GTCAAAGATA ATGAGAAGGT TAACTCTAAT GTTGCTGTAG CAAGGTCTCA GGGACGATAT 540
ACGACAAATG ATGGTTATGT CTTTAATCCA GCTGATATTA TCGAAGATAC GGGTAATGCT 600
TATATCGTTC CTCATGGAGG TCACTATCAC TACATTCCCA AAAGCGATTT ATCTGCTAGT 660
GAATTAGCAG CAGCTAAAGC ACATCTGGCT GGAAAAAATA TGCAACCGAG TCAGTTAAGC 720
TATTCTTCAA CAGCTAGTGA CAATAACACG CAATCTGTAG CAAAAGGATC AACTAGCAAG 780
CCAGCAAATA AATCTGAAAA TCTCCAGAGT CTTTTGAAGG AACTCTATGA TTCACCTAGC 840
GCCCAACGTT ACAGTGAATC AGATGGCCTG GTCTTTGACC CTGCTAAGAT TATCAGTCGT 900
ACACCAAATG GAGTTGCGAT TCCGCATGGC GACCATTACC ACTTTATTCC TTACAGCAAG 960
CTTTCTGCTT TAGAAGAAAA GATTGCCAGA ATGGTGCCTA TCAGTGGAAC TGGTTCTACA 1020
GTTTCTACAA ATGCAAAACC TAATGAAGTA GTGTCTAGTC TAGGCAGTCT TTCAAGCAAT 1080
CCTTCTTCTT TAACGACAAG TAAGGAGCTC TCTTCAGCAT CTGATGGTTA TATTTTTAAT 1140
CCAAAAGATA TCGTTGAAGA AACGGCTACA GCTTATATTG TAAGACATGG TGATCATTTC 1200
CATTACATTC CAAAATCAAA TCAAATTGGG CAACCGACTC TTCCAAACAA TAGTCTAGCA 1260
ACACCTTCTC CATCTCTTCC AATCAATCCA GGAACTTCAC ATGAGAAACA TGAAGAAGAT 1320
GGATACGGAT TTGATGCTAA TCGTATTATC GCTGAAGATG AATCAGGTTT TGTCATGAGT 1380
CACGGAGACC ACAATCATTA TTTCTTCAAG AAGGACTTGA CAGAAGAGCA AATTAAGGCT 1440
GCGCAAAAAC ATTTAGAGGA AGTTAAAACT AGTCATAATG GATTAGATTC TTTGTCATCT 1500
CATGAACAGG ATTATCCAGG TAATGCCAAA GAAATGAAAG ATTTAGATAA AAAAATCGAA 1560
GAAAAAATTG CTGGCATTAT GAAACAATAT GGTGTCAAAC GTGAAAGTAT TGTCGTGAAT 1620
AAAGAAAAAA ATGCGATTAT TTATCCGCAT GGAGATCACC ATCATGCAGA TCCGATTGAT 1680
GAACATAAAC CGGTTGGAAT TGGTCATTCT CACAGTAACT ATGAACTGTT TAAACCCGAA 1740
GAAGGAGTTG CTAAAAAAGA AGGGAATAAA GTTTATACTG GAGAAGAATT AACGAATGTT 1800
GTTAATTTGT TAAAAAATAG TACGTTTAAT AATCAAAACT TTACTCTAGC CAATGGTCAA 1860
AAACGCGTTT CTTTTAGTTT TCCGCCTGAA TTGGAGAAAA AATTAGGTAT CAATATGCTA 1920
GTAAAATTAA TAACACCAGA TGGAAAAGTA TTGGAGAAAG TATCTGGTAA AGTATTTGGA 1980
GAAGGAGTAG GGAATATTGC AAACTTTGAA TTAGATCAAC CTTATTTACC AGGACAAACA 2040
TTTAAGTATA CTATCGCTTC AAAAGATTAT CCAGAAGTAA GTTATGATGG TACATTTACA 2100
GTTCCAACCT CTTTAGCTTA CAAAATGGCC AGTCAAACGA TTTTCTATCC TTTCCATGCA 2160
GGGGATACTT ATTTAAGAGT GAACCCTCAA TTTGCAGTGC CTAAAGGAAC TGATGCTTTA 2220
GTCAGAGTGT TTGATGAATT TCATGGAAAT GCTTATTTAG AAAATAACTA TAAAGTTGGT 2280
GAAATCAAAT TACCGATTCC GAAATTAAAC CAAGGAACAA CCAGAACGGC CGGAAATAAA 2340
ATTCCTGTAA CCTTCATGGC AAATGCTTAT TTGGACAATC AATCGACTTA TATTGTGGAA 2400
GTACCTATCT TGGAAAAAGA AAATCAAACT GATAAACCAA GTATTCTACC ACAATTTAAA 2460
AGGAATAAAG CACAAGAAAA CTCAAAACTT GATGAAAAGG TAGAAGAACC AAAGACTAGT 2520
GAGAAGGTAG AAAAAGAAAA ACTTTCTGAA ACTGGGAATA GTACTAGTAA TTCAACGTTA 2580
GAAGAAGTTC CTACAGTGGA TCCTGTACAA GAAAAAGTAG CAAAATTTGC TGAAAGTTAT 2640
GGGATGAAGC TAGAAAATGT CTTGTTTAAT ATGGACGGAA CAATTGAATT ATATTTACCA 2700

TCAGGAGAAG TCATTAAAAA GAATATGGCA GATTTTACAG GAGAAGCACC TCAAGGAAAT 2760
GGTGAAAATA AACCATCTGA AAATGGAAAA GTATCTACTG GAACAGTTGA GAACCAACCA 2820
ACAGAAAATA AACCAGCAGA TTCTTTACCA GAGGCACCAA ACGAAAAACC TGTAAAACCA 2880
GAAAACTCAA CGGATAATGG AATGTTGAAT CCAGAAGGGA ATGTGGGGAG TGACCCTATG 2940
TTAGATCCAG CATTAGAGGA AGCTCCAGCA GTAGATCCTG TACAAGAAAA ATTAGAAAAA 3000
TTTACAGCTA GTTACGGATT AGGCTTAGAT AGTGTTATAT TCAATATGGA TGGAACGATT 3060
GAATTAAGAT TGCCAAGTGG AGAAGTGATA AAAAAGAATT TATCTGATTT CATAGCGTAA 3120
(SEQ ID NO: 1)
FIGURE 1

AATTCCTTGT CGGGTAAGTT CCGACCCGCA CGAAAGGCGT AATGATTTGG GCACTGTCTC 60
AACGAGAGAC TCGGTGAAAT TTTAGTACCT GTGAAGATGC AGGTTACCCG CGACAGGACG 120
GAAAGACCCC ATGGAGCTTT ACTGCAGTTT GATATTGAGT GTCTGTACCA CATGTACAGG 180
ATAGGTAGGA GTCTAAGAGA TCGGGACGCC AGTTTCGAAG GAGACGCTGT TGGGATACTA 240
CCCTTGTGTT ATGGCCACTC TAACCCAGAT AGGTGATCCC TATCGGAGAC AGTGTCTGAC 300
GGGCAGTTTG ACTGGGGCGG TCGCCTCCTA AAAGGTAACG GAGGCGCCCA AAGGTTCCCT 360
CAGAATGGTT GGAAATCATT CGCAGAGTGT AAAGGTATAA GGGAGCTTGA CTGCGAGAGC 420
TACAACTCGA GCAGGGACGA AAGTCGGGCT TAGTGATCCG GTGGTTCCGT ATGGAAGGGC 480
CATCGCTCAA CGGATAAAAG CTACCCTGGG GATAACAGGC TTATCTCCCC CAAGAGTTCA 540
CATCGACGGG GAGGTTTGGC ACCTCGATGT CGGCTCGTCG CATCCTGGGG CTGTAGTCGG 600
TCCCAAGGGT TGGGCTGTTC GCCCATTAAA GCGGCACGCG AGCTGGGTTC AGAACGTCGT 660
GAGACAGTTC GGTCCCTATC CGTCGCGGGC GTAGGAAATT TGAGAGGATC TGCTCCTAGT 720
ACGAGAGGAC CAGAGTGGAC TTACCGCTGG TGTACCAGTT GTCTTGCCAA AGGCATCGCT 780
GGGTAGCTAT GTAGGGAAGG GATAAACGCT GAAAGCATCT AAGTGTGAAA CCCACCTCAA 840
GATGAGATTT CCCATGATTA TATATCAGTA AGAGCCCTGA GAGATGATCA GGTAGATAGG 900
TTAGAAGTGG AAGTGTGGCG ACACATGTAG CGGACTAATA CTAATAGCTC GAGGACTTAT 960
CCAAAGTAAC TGAGAATATG AAAGCGAACG GTTTTCTTAA ATTGAATAGA TATTCAATTT 1020
TGAGTAGGTA TTACTCAGAG TTAAGTGACG ATAGCCTAGG AGATACACCT GTACCCATGC 1080
CGAACACAGA AGTTAAGCCC TAGAACGCCG GAAGTAGTTG GGGGTTGCCC CCTGTGAGAT 1140
AGGGAAGTCG CTTAGCTCTA GGGAGTTTAG CTCAGCTGGG AGAGCATCTG CCTTACAAGC 1200
AGAGGGTCAG CGGTTCGATC CCGTTAACTC CCAAAGGTCC CGTAGTGTAG CGGTTATCAC 1260
GTCGCCCTGT CACGGCGAAG ATCGCGGGTT CGATTCCCGT CGGGACCGTT TAAGGTAACG 1320
CAAGTTATTT TAGACTCGTT AGCTCAGTTG GTAGAGCAAT TGACTTTTAA TCAATGGGTC 1380
ACTGGTTCGA GCCCAGTACG GGTCATATAT GCGGGTTTGG CGGAATTCTA ATCTCTTTGA 1440
AATCATCTTC TCTCACTTTC CAAAACTCTA TTACCTCTTA TTATACCACA TTTCAATCTT 1500
CAACTTCCCA GTAATATAAG CACCTCTGGC GAAAGAAGTT TCAATGTCCT AAAGTAATAA 1560
GTGAATCCAA TTCAGGAACT CCAAGAACAA AAGAAACATC TGGTGTCACA AGTATTGGAT 1620
GGCACAGAGT CACGTGGTAG TCTGACCCTA GCAGAAATTT TAAATAGTAA ACTATTTACT 1680
GGTTAATTAA ATGGTTAAAT AACCGGTTTA GAAAACTATT TAATAAAGTA AAAGAAGTTG 1740
AGAAAAAACT TCATCATTTA TTGAAATGAG GGATTTATGA AATTTAGTAA AAAATATATA 1800
GCAGCTGGAT CAGCTGTTAT CGTATCCTTG AGTCTATGTG CCTATGCACT AAACCAGCAT 1860
CGTTCGCAGG AAAATAAGGA CAATAATCGT GTCTCTTATG TGGATGGCAG CCAGTCAAGT 1920
CAGAAAAGTG AAAACTTGAC ACCAGACCAG GTTAGCCAGA AAGAAGGAAT TCAGGCTGAG 1980
CAAATTGTAA TCAAAATTAC AGATCAGGGC TATGTAACGT CACACGGTGA CCACTATCAT 2040

TACTATAATG GGAAAGTTCC TTATGATGCC CTCTTTAGTG AAGAACTCTT GATGAAGGAT 2100
CCAAACTATC AACTTAAAGA CGCTGATATT GTCAATGAAG TCAAGGGTGG TTATATCATC 2160
AAGGTCGATG GAAAATATTA TGTCTACCTG AAAGATGCAG CTCATGCTGA TAATGTTCGA 2220
ACTAAAGATG AAATCAATCG TCAAAAACAA GAACATGTCA AAGATAATGA GAAGGTTAAC 2280
TCTAATGTTG CTGTAGCAAG GTCTCAGGGA CGATATACGA CAAATGATGG TTATGTCTTT 2340
AATCCAGCTG ATATTATCGA AGATACGGGT AATGCTTATA TCGTTCCTCA TGGAGGTCAC 2400
TATCACTACA TTCCCAAAAG CGATTTATCT GCTAGTGAAT TAGCAGCAGC TAAAGCACAT 2460
CTGGCTGGAA AAAATATGCA ACCGAGTCAG TTAAGCTATT CTTCAACAGC TAGTGACAAT 2520
AACACGCAAT CTGTAGCAAA AGGATCAACT AGCAAGCCAG CAAATAAATC TGAAAATCTC 2580
CAGAGTCTTT TGAAGGAACT CTATGATTCA CCTAGCGCCC AACGTTACAG TGAATCAGAT 2640
GGCCTGGTCT TTGACCCTGC TAAGATTATC AGTCGTACAC CAAATGGAGT TGCGATTCCG 2700
CATGGCGACC ATTACCACTT TATTCCTTAC AGCAAGCTTT CTGCTTTAGA AGAAAAGATT 2760
GCCAGAATGG TGCCTATCAG TGGAACTGGT TCTACAGTTT CTACAAATGC AAAACCTAAT 2820
GAAGTAGTGT CTAGTCTAGG CAGTCTTTCA AGCAATCCTT CTTCTTTAAC GACAAGTAAG 2880
GAGCTCTCTT CAGCATCTGA TGGTTATATT TTTAATCCAA AAGATATCGT TGAAGAAACG 2940
GCTACAGCTT ATATTGTAAG ACATGGTGAT CATTTCCATT ACATTCCAAA ATCAAATCAA 3000
ATTGGGCAAC CGACTCTTCC AAACAATAGT CTAGCAACAC CTTCTCCATC TCTTCCAATC 3060
AATCCAGGAA CTTCACATGA GAAACATGAA GAAGATGGAT ACGGATTTGA TGCTAATCGT 3120
ATTATCGCTG AAGATGAATC AGGTTTTGTC ATGAGTCACG GAGACCACAA TCATTATTTC 3180
TTCAAGAAGG ACTTGACAGA AGAGCAAATT AAGGCTGCGC AAAAACATTT AGAGGAAGTT 3240
AAAACTAGTC ATAATGGATT AGATTCTTTG TCATCTCATG AACAGGATTA TCCAGGTAAT 3300
GCCAAAGAAA TGAAAGATTT AGATAAAAAA ATCGAAGAAA AAATTGCTGG CATTATGAAA 3360
CAATATGGTG TCAAACGTGA AAGTATTGTC GTGAATAAAG AAAAAAATGC GATTATTTAT 3420
CCGCATGGAG ATCACCATCA TGCAGATCCG ATTGATGAAC ATAAACCGGT TGGAATTGGT 3480
CATTCTCACA GTAACTATGA ACTGTTTAAA CCCGAAGAAG GAGTTGCTAA AAAAGAAGGG 3540
AATAAAGTTT ATACTGGAGA AGAATTAACG AATGTTGTTA ATTTGTTAAA AAATAGTACG 3600
TTTAATAATC AAAACTTTAC TCTAGCCAAT GGTCAAAAAC GCGTTTCTTT TAGTTTTCCG 3660
CCTGAATTGG AGAAAAAATT AGGTATCAAT ATGCTAGTAA AATTAATAAC ACCAGATGGA 3720
AAAGTATTGG AGAAAGTATC TGGTAAAGTA TTTGGAGAAG GAGTAGGGAA TATTGCAAAC 3780
TTTGAATTAG ATCAACCTTA TTTACCAGGA CAAACATTTA AGTATACTAT CGCTTCAAAA 3840
GATTATCCAG AAGTAAGTTA TGATGGTACA TTTACAGTTC CAACCTCTTT AGCTTACAAA 3900
ATGGCCAGTC AAACGATTTT CTATCCTTTC CATGCAGGGG ATACTTATTT AAGAGTGAAC 3960
CCTCAATTTG CAGTGCCTAA AGGAACTGAT GCTTTAGTCA GAGTGTTTGA TGAATTTCAT 4020
GGAAATGCTT ATTTAGAAAA TAACTATAAA GTTGGTGAAA TCAAATTACC GATTCCGAAA 4080
TTAAACCAAG GAACAACCAG AACGGCCGGA AATAAAATTC CTGTAACCTT CATGGCAAAT 4140
GCTTATTTGG ACAATCAATC GACTTATATT GTGGAAGTAC CTATCTTGGA AAAAGAAAAT 4200
CAAACTGATA AACCAAGTAT TCTACCACAA TTTAAAAGGA ATAAAGCACA AGAAAACTCA 4260
AAACTTGATG AAAAGGTAGA AGAACCAAAG ACTAGTGAGA AGGTAGAAAA AGAAAAACTT 4320
TCTGAAACTG GGAATAGTAC TAGTAATTCA ACGTTAGAAG AAGTTCCTAC AGTGGATCCT 4380
GTACAAGAAA AAGTAGCAAA ATTTGCTGAA AGTTATGGGA TGAAGCTAGA AAATGTCTTG 4440
TTTAATATGG ACGGAACAAT TGAATTATAT TTACCATCAG GAGAAGTCAT TAAAAAGAAT 4500
ATGGCAGATT TTACAGGAGA AGCACCTCAA GGAAATGGTG AAAATAAACC ATCTGAAAAT 4560
GGAAAAGTAT CTACTGGAAC AGTTGAGAAC CAACCAACAG AAAATAAACC AGCAGATTCT 4620
TTACCAGAGG CACCAAACGA AAAACCTGTA AAACCAGAAA ACTCAACGGA TAATGGAATG 4680
TTGAATCCAG AAGGGAATGT GGGGAGTGAC CCTATGTTAG ATCCAGCATT AGAGGAAGCT 4740

CCAGCAGTAG ATCCTGTACA AGAAAAATTA GAAAAATTTA CAGCTAGTTA CGGATTAGGC 4800
TTAGATAGTG TTATATTCAA TATGGATGGA ACGATTGAAT TAAGATTGCC AAGTGGAGAA 4860
GTGATAAAAA AGAATTTATC TGATTTCATA GCGTAAGGAA TAGCAGTAGA AAAAGTCTGA 4920
ATCAAAAATG AAGTTCTCTC AAAAGTTAGA AATAAAACTC TGACTTTGGG AGAATTTCAT 4980
TTTATTATTA ATATATAAAA TTTCTTGACA TACAACTTAA AAAGAGGTGG AATATTTACT 5040
AGTTAATT (SEQ ID NO : 2) 5048

FIGURE 2

ATGAAAATCA ATAAAAAATA TCTAGCTGGG TCAGTAGCTA CACTTGTTTT AAGTGTCTGT 60
GCTTATGAAC TAGGTTTGCA TCAAGCTCAA ACTGTAAAAG AAAATAATCG TGTTTCCTAT 120
ATAGATGGAA AACAAGCGAC GCAAAAAACG GAGAATTTGA CTCCTGATGA GGTTAGCAAG 180
CGTGAAGGAA TCAACGCCGA ACAAATCGTC ATCAAGATTA CGGATCAAGG TTATGTGACC 240
TCTCATGGAG ACCATTATCA TTACTATAAT GGCAAGGTCC CTTATGATGC CATCATCAGT 300
GAAGAGCTCC TCATGAAAGA TCCGAATTAT CAGTTGAAGG ATTCAGACAT TGTCAATGAA 360
ATCAAGGGTG GTTATGTCAT TAAGGTAAAC GGTAAATACT ATGTTTACCT TAAGGATGCA 420
GCTCATGCGG ATAATGTCCG TACAAAAGAA GAAATCAATC GGCAAAAACA AGAACATAGT 480
CAGCATCGTG AAGGAGGGAC TTCAGCAAAC GATGGTGCGG TAGCCTTTGC ACGTTCACAG 540
GGACGCTACA CCACAGATGA TGGTTATATC TTCAATGCAT CTGATATCAT CGAAGATACG 600
GGCGATGCCT ATATCGTTCC TCATGGAGAT CATTACCATT ACATTCCTAA GAATGAGTTA 660
TCAGCTAGCG AGTTGGCTGC TGCAGAAGCC TTCCTATCTG GTCGGGAAAA TCTGTCAAAT 720
TTAAGAACCT ATCGCCGACA AAATAGCGAT AACACTCCAA GAACAAACTG GGTACCTTCT 780
GTAAGCAATC CAGGAACTAC AAATACTAAC ACAAGCAACA ACAGCAACAC TAACAGTCAA 840
GCAAGTCAAA GTAATGACAT TGATAGTCTC TTGAAACAGC TCTACAAACT GCCTTTGAGT 900
CAACGCCATG TAGAATCTGA TGGCCTTATT TTCGACCCAG CGCAAATCAC AAGTCGAACC 960
GCCAGAGGTG TAGCTGTCCC TCATGGTAAC CATTACCACT TTATCCCTTA TGAACAAATG 1020
TCTGAATTGG AAAAACGAAT TGCTCGTATT ATTCCCCTTC GTTATCGTTC AAACCATTGG 1080
GTACCAGATT CAAGACCAGA AGAACCAAGT CCACAACCGA CTCCAGAACC TAGTCCAAGT 1140
CCGCAACCTG CACCAAATCC TCAACCAGCT CCAAGCAATC CAATTGATGA GAAATTGGTC 1200
AAAGAAGCTG TTCGAAAAGT AGGCGATGGT TATGTCTTTG AGGAGAATGG AGTTTCTCGT 1260
TATATCCCAG CCAAGAATCT TTCAGCAGAA ACAGCAGCAG GCATTGATAG CAAACTGGCC 1320
AAGCAGGAAA GTTTATCTCA TAAGCTAGGA GCTAAGAAAA CTGACCTCCC ATCTAGTGAT 1380
CGAGAATTTT ACAATAAGGC TTATGACTTA CTAGCAAGAA TTCACCAAGA TTTACTTGAT 1440
AATAAAGGTC GACAAGTTGA TTTTGAGGCT TTGGATAACC TGTTGGAACG ACTCAAGGAT 1500
GTCTCAAGTG ATAAAGTCAA GTTAGTGGAT GATATTCTTG CCTTCTTAGC TCCGATTCGT 1560
CATCCAGAAC GTTTAGGAAA ACCAAATGCG CAAATTACCT ACACTGATGA TGAGATTCAA 1620
GTAGCCAAGT TGGCAGGCAA GTACACAACA GAAGACGGTT ATATCTTTGA TCCTCGTGAT 1680
ATAACCAGTG ATGAGGGGGA TGCCTATGTA ACTCCACATA TGACCCATAG CCACTGGATT 1740
AAAAAAGATA GTTTGTCTGA AGCTGAGAGA GCGGCAGCCC AGGCTTATGC TAAAGAGAAA 1800
GGTTTGACCC CTCCTTCGAC AGACCATCAG GATTCAGGAA ATACTGAGGC AAAAGGAGCA 1860
GAAGCTATCT ACAACCGCGT GAAAGCAGCT AAGAAGGTGC CACTTGATCG TATGCCTTAC 1920
AATCTTCAAT ATACTGTAGA AGTCAAAAAC GGTAGTTTAA TCATACCTCA TTATGACCAT 1980
TACCATAACA TCAAATTTGA GTGGTTTGAC GAAGGCCTTT ATGAGGCACC TAAGGGGTAT 2040
ACTCTTGAGG ATCTTTTGGC GACTGTCAAG TACTATGTCG AACATCCAAA CGAACGTCCG 2100
CATTCAGATA ATGGTTTTGG TAACGCTAGC GACCATGTTC AAAGAAACAA AAATGGTCAA 2160

GCTGATACCA ATCAAACGGA AAAACCAAGC GAGGAGAAAC CTCAGACAGA AAAACCTGAG 2220
GAAGAAACCC CTCGAGAAGA GAAACCACAA AGCGAGAAAC CAGAGTCTCC AAAACCAACA 2280
GAGGAACCAG AAGAAGAATC ACCAGAGGAA TCAGAAGAAC CTCAGGTCGA GACTGAAAAG 2340
GTTGAAGAAA AACTGAGAGA GGCTGAAGAT TTACTTGGAA AAATCCAGGA TCCAATTATC 2400
AAGTCCAATG CCAAAGAGAC TCTCACAGGA TTAAAAAATA ATTTACTATT TGGCACCCAG 2460
GACAACAATA CTATTATGGC AGAAGCTGAA AAACTATTGG CTTTATTAAA GGAGAGTAAG 2520
TAA (SEQ ID NO: 3) 2523
FIGURE 3

CAGAGATCTT AGTGAATCAA ATATACTTAA GAAAAGAGGA AAGAATGAAA ATCAATAAAA 60
AATATCTAGC TGGGTCAGTA GCTACACTTG TTTTAAGTGT CTGTGCTTAT GAACTAGGTT 120
TGCATCAAGC TCAAACTGTA AAAGAAAATA ATCGTGTTTC CTATATAGAT GGAAAACAAG 180
CGACGCAAAA AACGGAGAAT TTGACTCCTG ATGAGGTTAG CAAGCGTGAA GGAATCAACG 240
CCGAACAAAT CGTCATCAAG ATTACGGATC AAGGTTATGT GACCTCTCAT GGAGACCATT 300
ATCATTACTA TAATGGCAAG GTCCCTTATG ATGCCATCAT CAGTGAAGAG CTCCTCATGA 360
AAGATCCGAA TTATCAGTTG AAGGATTCAG ACATTGTCAA TGAAATCAAG GGTGGTTATG 420
TCATTAAGGT AAACGGTAAA TACTATGTTT ACCTTAAGGA TGCAGCTCAT GCGGATAATG 480
TCCGTACAAA AGAAGAAATC AATCGGCAAA AACAAGAACA TAGTCAGCAT CGTGAAGGAG 540
GGACTTCAGC AAACGATGGT GCGGTAGCCT TTGCACGTTC ACAGGGACGC TACACCACAG 600
ATGATGGTTA TATCTTCAAT GCATCTGATA TCATCGAAGA TACGGGCGAT GCCTATATCG 660
TTCCTCATGG AGATCATTAC CATTACATTC CTAAGAATGA GTTATCAGCT AGCGAGTTGG 720
CTGCTGCAGA AGCCTTCCTA TCTGGTCGGG AAAATCTGTC AAATTTAAGA ACCTATCGCC 780
GACAAAATAG CGATAACACT CCAAGAACAA ACTGGGTACC TTCTGTAAGC AATCCAGGAA 840
CTACAAATAC TAACACAAGC AACAACAGCA ACACTAACAG TCAAGCAAGT CAAAGTAATG 900
ACATTGATAG TCTCTTGAAA CAGCTCTACA AACTGCCTTT GAGTCAACGC CATGTAGAAT 960
CTGATGGCCT TATTTTCGAC CCAGCGCAAA TCACAAGTCG AACCGCCAGA GGTGTAGCTG 1020
TCCCTCATGG TAACCATTAC CACTTTATCC CTTATGAACA AATGTCTGAA TTGGAAAAAC 1080
GAATTGCTCG TATTATTCCC CTTCGTTATC GTTCAAACCA TTGGGTACCA GATTCAAGAC 1140
CAGAAGAACC AAGTCCACAA CCGACTCCAG AACCTAGTCC AAGTCCGCAA CCTGCACCAA 1200
ATCCTCAACC AGCTCCAAGC AATCCAATTG ATGAGAAATT GGTCAAAGAA GCTGTTCGAA 1260
AAGTAGGCGA TGGTTATGTC TTTGAGGAGA ATGGAGTTTC TCGTTATATC CCAGCCAAGA 1320
ATCTTTCAGC AGAAACAGCA GCAGGCATTG ATAGCAAACT GGCCAAGCAG GAAAGTTTAT 1380
CTCATAAGCT AGGAGCTAAG AAAACTGACC TCCCATCTAG TGATCGAGAA TTTTACAATA 1440
AGGCTTATGA CTTACTAGCA AGAATTCACC AAGATTTACT TGATAATAAA GGTCGACAAG 1500
TTGATTTTGA GGCTTTGGAT AACCTGTTGG AACGACTCAA GGATGTCTCA AGTGATAAAG 1560
TCAAGTTAGT GGATGATATT CTTGCCTTCT TAGCTCCGAT TCGTCATCCA GAACGTTTAG 1620
GAAAACCAAA TGCGCAAATT ACCTACACTG ATGATGAGAT TCAAGTAGCC AAGTTGGCAG 1680
GCAAGTACAC AACAGAAGAC GGTTATATCT TTGATCCTCG TGATATAACC AGTGATGAGG 1740
GGGATGCCTA TGTAACTCCA CATATGACCC ATAGCCACTG GATTAAAAAA GATAGTTTGT 1800
CTGAAGCTGA GAGAGCGGCA GCCCAGGCTT ATGCTAAAGA GAAAGGTTTG ACCCCTCCTT 1860
CGACAGACCA TCAGGATTCA GGAAATACTG AGGCAAAAGG AGCAGAAGCT ATCTACAACC 1920
GCGTGAAAGC AGCTAAGAAG GTGCCACTTG ATCGTATGCC TTACAATCTT CAATATACTG 1980
TAGAAGTCAA AAACGGTAGT TTAATCATAC CTCATTATGA CCATTACCAT AACATCAAAT 2040
TTGAGTGGTT TGACGAAGGC CTTTATGAGG CACCTAAGGG GTATACTCTT GAGGATCTTT 2100

TGGCGACTGT CAAGTACTAT GTCGAACATC CAAACGAACG TCCGCATTCA GATAATGGTT 2160
TTGGTAACGC TAGCGACCAT GTTCAAAGAA ACAAAAATGG TCAAGCTGAT ACCAATCAAA 2220
CGGAAAAACC AAGCGAGGAG AAACCTCAGA CAGAAAAACC TGAGGAAGAA ACCCCTCGAG 2280
AAGAGAAACC ACAAAGCGAG AAACCAGAGT CTCCAAAACC AACAGAGGAA CCAGAAGAAG 2340
AATCACCAGA GGAATCAGAA GAACCTCAGG TCGAGACTGA AAAGGTTGAA GAAAAACTGA 2400
GAGAGGCTGA AGATTTACTT GGAAAAATCC AGGATCCAAT TATCAAGTCC AATGCCAAAG 2460
AGACTCTCAC AGGATTAAAA AATAATTTAC TATTTGGCAC CCAGGACAAC AATACTATTA 2520
TGGCAGAAGC TGAAAAACTA TTGGCTTTAT TAAAGGAGAG TAAGTAAAGG TAGCAGCATT 2580
TTCTAACTCC TAAAAACAGG ATAGGAGAAC GGGAAAACGA AAAATGAGAG CAGAATGTGA 2640
GTTCTAG (SED ID NO : 4) 2647
FIGURE 4

GGGTCTTAAA ACTCTGAATC CTTTAGAGGC AGACCCACAA AATGACAAGA CCTATTTAGA 60
AAATCTGGAA GAAAATATGA GTGTTCTAGC AGAAGAATTA AAGTGAGGAA AGAATGAAAA 120
TCAATAAAAA ATATCTAGCA GGTTCAGTGG CAGTCCTTGC CCTAAGTGTT TGTTCCTATG 180
AACTTGGTCG TCACCAAGCT GGTCAGGTTA AGAAAGAGTC TAATCGAGTT TCTTATATAG 240
ATGGTGATCA GGCTGGTCAA AAGGCAGAAA ATTTGACACC AGATGAAGTC AGTAAGAGAG 300
AGGGGATCAA CGCCGAACAA ATTGTTATCA AGATTACGGA TCAAGGTTAT GTGACCTCTC 360
ATGGAGACCA TTATCATTAC TATAATGGCA AGGTTCCTTA TGATGCCATC ATCAGTGAAG 420
AACTTCTCAT GAAAGATCCG AATTATCAGT TGAAGGATTC AGACATTGTC AATGAAATCA 480
AGGGTGGCTA TGTGATTAAG GTAGACGGAA AATACTATGT TTACCTTAAA GATGCGGCCC 540
ATGCGGACAA TATTCGGACA AAAGAAGAGA TTAAACGTCA GAAGCAGGAA CACAGTCATA 600
ATCATAACTC AAGAGCAGAT AATGCTGTTG CTGCAGCCAG AGCCCAAGGA CGTTATACAA 660
CGGATGATGG GTATATCTTC AATGCATCTG ATATCATTGA GGACACGGGT GATGCTTATA 720
TCGTTCCTCA CGGCGACCAT TACCATTACA TTCCTAAGAA TGAGTTATCA GCTAGCGAGT 780
TAGCTGCTGC AGAAGCCTAT TGGAATGGGA AGCAGGGATC TCGTCCTTCT TCAAGTTCTA 840
GTTATAATGC AAATCCAGTT CAACCAAGAT TGTCAGAGAA CCACAATCTG ACTGTCACTC 900
CAACTTATCA TCAAAATCAA GGGGAAAACA TTTCAAGCCT TTTACGTGAA TTGTATGCTA 960
AACCCTTATC AGAACGCCAT GTAGAATCTG ATGGCCTTAT TTTCGACCCA GCGCAAATCA 1020
CAAGTCGAAC CGCCAGAGGT GTAGCTGTCC CTCATGGTAA CCATTACCAC TTTATCCCTT 1080
ATGAACAAAT GTCTGAATTG GAAAAACGAA TTGCTCGTAT TATTCCCCTT CGTTATCGTT 1140
CAAACCATTG GGTACCAGAT TCAAGACCAG AACAACCAAG TCCACAATCG ACTCCGGAAC 1200
CTAGTCCAAG TCTGCAACCT GCACCAAATC CTCAACCAGC TCCAAGCAAT CCAATTGATG 1260
AGAAATTGGT CAAAGAAGCT GTTCGAAAAG TAGGCGATGG TTATGTCTTT GAGGAGAATG 1320
GAGTTTCTCG TTATATCCCA GCCAAGGATC TTTCAGCAGA AACAGCAGCA GGCATTGATA 1380
GCAAACTGGC CAAGCAGGAA AGTTTATCTC ATAAGCTAGG AGCTAAGAAA ACTGACCTCC 1440
CATCTAGTGA TCGAGAATTT TACAATAAGG CTTATGACTT ACTAGCAAGA ATTCACCAAG 1500
ATTTACTTGA TAATAAAGGT CGACAAGTTG ATTTTGAGGT TTTGGATAAC CTGTTGGAAC 1560
GACTCAAGGA TGTCTCAAGT GATAAAGTCA AGTTAGTGGA TGATATTCTT GCCTTCTTAG 1620
CTCCGATTCG TCATCCAGAA CGTTTAGGAA AACCAAATGC GCAAATTACC TACACTGATG 1680
ATGAGATTCA AGTAGCCAAG TTGGCAGGCA AGTACACAAC AGAAGACGGT TATATCTTTG 1740
ATCCTCGTGA TATAACCAGT GATGAGGGGG ATGCCTATGT AACTCCACAT ATGACCCATA 1800
GCCACTGGAT TAAAAAAGAT AGTTTGTCTG AAGCTGAGAG AGCGGCAGCC CAGGCTTATG 1860
CTAAAGAGAA AGGTTTGACC CCTCCTTCGA CAGACCACCA GGATTCAGGA AATACTGAGG 1920

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CAAAAGGAGC AGAAGCTATC TACAACCGCG TGAAAGCAGC TAAGAAGGTG CCACTTGATC    1980
GTATGCCTTA CAATCTTCAA TATACTGTAG AAGTCAAAAA CGGTAGTTTA ATCATACCTC    2040
ATTATGACCA TTACCATAAC ATCAAATTTG AGTGGTTTGA CGAAGGCCTT TATGAGGCAC    2100
CTAAGGGGTA TAGTCTTGAG GATCTTTTGG CGACTGTCAA GTACTATGTC GAACATCCAA    2160
ACGAACGTCC GCATTCAGAT AATGGTTTTG GTAACGCTAG TGACCATGTT CGTAAAAATA    2220
AGGCAGACCA AGATAGTAAA CCTGATGAAG ATAAGGAACA TGATGAAGTA AGTGAGCCAA    2280
CTCACCCTGA ATCTGATGAA AAAGAGAATC ACGCTGGTTT AAATCCTTCA GCAGATAATC    2340
TTTATAAACC AAGCACTGAT ACGGAAGAGA CAGAGGAAGA AGCTGAAGAT ACCACAGATG    2400
AGGCTGAAAT TCCTCAAGTA GAGAATTCTG TTATTAACGC TAAGATAGCA GATGCGGAGG    2460
CCTTGCTAGA AAAAGTAACA GATCCTAGTA TTAGACAAAA TGCTATGGAG ACATTGACTG    2520
GTCTAAAAAG TAGTCTTCTT CTCGGAACGA AAGATAATAA CACTATTTCA GCAGAAGTAG    2580
ATAGTCTCTT GGCTTTGTTA AAAGAAAGTC AACCGGCTCC TATACAGTAG TAAAATGAA     2639
(SEQ ID NO : 5)
FIGURE 5
```

```
MKFSKKYIAA GSAVIVSLSL CAYALNQHRS QENKDNNRVS YVDGSQSSQK      50
SENLTPDQVS QKEGIQAEQI VIKITDQGYV TSHGDHYHYY NGKVPYDALF     100
SEELLMKDPN YQLKDADIVN EVKGGYIIKV DGKYYVYLKD AAHADNVRTK     150
DEINRQKQEH VKDNEKVNSN VAVARSQGRY TTNDGYVFNP ADIIEDTGNA     200
YIVPHGGHYH YIPKSDLSAS ELAAAKAHLA GKNMQPSQLS YSSTASDNNT     250
QSVAKGSTSK PANKSENLQS LLKELYDSPS AQRYSESDGL VFDPAKIISR     300
TPNGVAIPHG DHYHFIPYSK LSALEEKIAR MVPISGTGST VSTNAKPNEV     350
VSSLGSLSSN PSSLTTSKEL SSASDGYIFN PKDIVEETAT AYIVRHGDHF     400
HYIPKSNQIG QPTLPNNSLA TPSPSLPINP GTSHEKHEED GYGFDANRII     450
AEDESGFVMS HGDHNHYFFK KDLTEEQIKA AQKHLEEVKT SHNGLDSLSS     500
HEQDYPGNAK EMKDLDKKIE EKIAGIMKQY GVKRESIVVN KEKNAIIYPH     550
GDHHHADPID EHKPVGIGHS HSNYELFKPE EGVAKKEGNK VYTGEELTNV     600
VNLLKNSTFN NQNFTLANGQ KRVSFSFPPE LEKKLGINML VKLITPDGKV     650
LEKVSGKVFG EGVGNIANFE LDQPYLPGQT FKYTIASKDY PEVSYDGTFT     700
VPTSLAYKMA SQTIFYPFHA GDTYLRVNPQ FAVPKGTDAL VRVFDEFHGN     750
AYLENNYKVG EIKLPIPKLN QGTTRTAGNK IPVTFMANAY LDNQSTYIVE     800
VPILEKENQT DKPSILPQFK RNKAQENSKL DEKVEEPKTS EKVEKEKLSE     850
TGNSTSNSTL EEVPTVDPVQ EKVAKFAESY GMKLENVLFN MDGTIELYLP     900
SGEVIKKNMA DFTGEAPQGN GENKPSENGK VSTGTVENQP TENKPADSLP     950
EAPNEKPVKP ENSTDNGMLN PEGNVGSDPM LDPALEEAPA VDPVQEKLEK    1000
FTASYGLGLD SVIFNMDGTI ELRLPSGEVI KKNLSDFIA (SEQ ID NO: 6) 1039
FIGURE 6
```

```
MKINKKYLAG SVATLVLSVC AYELGLHQAQ TVKENNRVSY IDGKQATQKT      50
ENLTPDEVSK REGINAEQIV IKITDQGYVT SHGDHYHYYN GKVPYDAIIS     100
EELLMKDPNY QLKDSDIVNE IKGGYVIKVN GKYYVYLKDA AHADNVRTKE     150
EINRQKQEHS QHREGGTSAN DGAVAFARSQ GRYTTDDGYI FNASDIIEDT     200
GDAYIVPHGD HYHYIPKNEL SASELAAAEA FLSGRENLSN LRTYRRQNSD     250
```

```
NTPRTNWVPS VSNPGTTNTN TSNNSNTNSQ ASQSNDIDSL LKQLYKLPLS      300
QRHVESDGLI FDPAQITSRT ARGVAVPHGN HYHFIPYEQM SELEKRIARI      350
IPLRYRSNHW VPDSRPEEPS PQPTPEPSPS PQPAPNPQPA PSNPIDEKLV      400
KEAVRKVGDG YVFEENGVSR YIPAKNLSAE TAAGIDSKLA KQESLSHKLG      450
AKKTDLPSSD REFYNKAYDL LARIHQDLLD NKGRQVDFEA LDNLLERLKD      500
VSSDKVKLVD DILAFLAPIR HPERLGKPNA QITYTDDEIQ VAKLAGKYTT      550
EDGYIFDPRD ITSDEGDAYV TPHMTHSHWI KKDSLSEAER AAAQAYAKEK      600
GLTPPSTDHQ DSGNTEAKGA EAIYNRVKAA KKVPLDRMPY NLQYTVEVKN      650
GSLIIPHYDH YHNIKFEWFD EGLYEAPKGY TLEDLLATVK YYVEHPNERP      700
HSDNGFGNAS DHVQRNKNGQ ADTNQTEKPS EEKPQTEKPE EETPREEKPQ      750
SEKPESPKPT EEPEEESPEE SEEPQVETEK VEEKLREAED LLGKIQDPII      800
KSNAKETLTG LKNNLLFGTQ DNNTIMAEAE KLLALLKESK (SEQ ID NO: 7)  840
```

FIGURE 7

```
MKINKKYLAG SVAVLALSVC SYELGRHQAG QVKKESNRVS YIDGDQAGQK       50
AENLTPDEVS KREGINAEQI VIKITDQGYV TSHGDHYHYY NGKVPYDAII      100
SEELLMKDPN YQLKDSDIVN EIKGGYVIKV DGKYYVYLKD AAHADNIRTK      150
EEIKRQKQEH SHNHNSRADN AVAAARAQGR YTTDDGYIFN ASDIIEDTGD      200
AYIVPHGDHY HYIPKNELSA SELAAAEAYW NGKQGSRPSS SSSYNANPVQ      250
PRLSENHNLT VTPTYHQNQG ENISSLLREL YAKPLSERHV ESDGLIFDPA      300
QITSRTARGV AVPHGNHYHF IPYEQMSELE KRIARIIPLR YRSNHWVPDS      350
RPEQPSPQST PEPSPSLQPA PNPQPAPSNP IDEKLVKEAV RKVGDGYVFE      400
ENGVSRYIPA KDLSAETAAG IDSKLAKQES LSHKLGAKKT DLPSSDREFY      450
NKAYDLLARI HQDLLDNKGR QVDFEVLDNL LERLKDVSSD KVKLVDDILA      500
FLAPIRHPER LGKPNAQITY TDDEIQVAKL AGKYTTEDGY IFDPRDITSD      550
EGDAYVTPHM THSHWIKKDS LSEAERAAAQ AYAKEKGLTP PSTDHQDSGN      600
TEAKGAEAIY NRVKAAKKVP LDRMPYNLQY TVEVKNGSLI IPHYDHYHNI      650
KFEWFDEGLY EAPKGYSLED LLATVKYYVE HPNERPHSDN GFGNASDHVR      700
KNKADQDSKP DEDKEHDEVS EPTHPESDEK ENHAGLNPSA DNLYKPSTDT      750
EETEEEAEDT TDEAEIPQVE NSVINAKIAD AEALLEKVTD PSIRQNAMET      800
LTGLKSSLLL GTKDNNTISA EVDSLLALLK ESQPAPIQ                   838
(SEQ ID NO : 8)
```

FIGURE 8

```
TGTGCCTATG CACTAAACCA GCATCGTTCG CAGGAAAATA AGGACAATAA TCGTGTCTCT       60
TATGTGGATG GCAGCCAGTC AAGTCAGAAA AGTGAAAACT TGACACCAGA CCAGGTTAGC      120
CAGAAAGAAG GAATTCAGGC TGAGCAAATT GTAATCAAAA TTACAGATCA GGGCTATGTA      180
ACGTCACACG GTGATCACTA TCATTACTAT AATGGGAAAG TTCCTTATGA TGCCCTCTTT      240
AGTGAAGAAC TCTTGATGAA GGATCCAAAC TATCAACTTA AAGACGCTGA TATTGTCAAT      300
GAAGTCAAGG GTGGTTATAT CATCAAGGTC GATGGAAAAT ATTATGTCTA CCTGAAAGAT      360
GCAGCTCATG CTGATAATGT TCGAACTAAA GATGAAATCA ATCGTCAAAA ACAAGAACAT      420
GTCAAAGATA ATGAGAAGGT TAACTCTAAT GTTGCTGTAG CAAGGTCTCA GGGACGATAT      480
ACGACAAATG ATGGTTATGT CTTTAATCCA GCTGATATTA TCGAAGATAC GGGTAATGCT      540
```

```
TATATCGTTC CTCATGGAGG TCACTATCAC TACATTCCCA AAAGCGATTT ATCTGCTAGT      600
GAATTAGCAG CAGCTAAAGC ACATCTGGCT GGAAAAAATA TGCAACCGAG TCAGTTAAGC      660
TATTCTTCAA CACCTTCTCC ATCTCTTCCA ATCAATCCAG GAACTTCACA TGAGAAACAT      720
GAAGAAGATG GATACGGATT TGATGCTAAT CGTATTATCG CTGAAGATGA ATCAGGTTTT      780
GTCATGAGTC ACGGAGACCA CAATCATTAT TTCTTCAAGA AGGACTTGAC AGAAGAGCAA      840
ATTAAGGCTG CGCAAAAACA TTTAGAGGAA GTTAAAACTA GTCATAATGG ATTAGATTCT      900
TTGTCATCTC ATGAACAGGA TTATCCAAGT AATGCCAAAG AAATGAAAGA TTTAGATAAA      960
AAAATCGAAG AAAAAATTGC TGGCATTATG AAACAATATG GTGTCAAACG TGAAAGTATT     1020
GTCGTGAATA AAGAAAAAAA TGCGATTATT TATCCGCATG GAGATCACCA TCATGCAGAT     1080
CCGATTGATG AACATAAACC GGTTGGAATT GGTCATTCTC ACAGTAACTA TGAACTGTTT     1140
AAACCCGAAG AAGGAGTTGC TAAAAAAGAA GGGAATAAAG TTTATACTGG AGAAGAATTA     1200
ACGAATGTTG TTAATTTGTT AAAAAATAGT ACGTTTAATA ATCAAAACTT TACTCTAGCC     1260
AATGGTCAAA AACGCGTTTC TTTTAGTTTT CCGCCTGAAT TGGAGAAAAA ATTAGGTATC     1320
AATATGCTAG TAAAATTAAT AACACCAGAT GGAAAAGTAT TGGAGAAAGT ATCTGGTAAA     1380
GTATTTGGAG AAGGAGTAGG GAATATTGCA AACTTTGAAT TAGATCAACC TTATTTACCA     1440
GGACAAACAT TTAAGTATAC TATCGCTTCA AAAGATTATC CAGAAGTAAG TTATGATGGT     1500
ACATTTACAG TTCCAACCTC TTTAGCTTAC AAAATGGCCA GTCAAACGAT TTTCTATCCT     1560
TTCCATGCAG GGGATACTTA TTTAAGAGTG AACCCTCAAT TTGCAGTGCC TAAAGGAACT     1620
GATGCTTTAG TCAGAGTGTT TGATGAATTT CATGGAAATG CTTATTTAGA AAATAACTAT     1680
AAAGTTGGTG AAATCAAATT ACCGATTCCG AAATTAAACC AAGGAACAAC CAGAACGGCC     1740
GGAAATAAAA TTCCTGTAAC CTTCATGGCA AATGCTTATT TGGACAATCA ATCGACTTAT     1800
ATTGTGGAAG TACCTATCTT GGAAAAAGAA AATCAAACTG ATAAACCAAG TATTCTACCA     1860
CAATTTAAAA GGAATAAAGC ACAAGAAAAC TCAAAACTTG ATGAAAAGGT AGAAGAACCA     1920
AAGACTAGTG AGAAGGTAGA AAAAGAAAAA CTTTCTGAAA CTGGGAATAG TACTAGTAAT     1980
TCAACGTTAG AAGAAGTTCC TACAGTGGAT CCTGTACAAG AAAAAGTAGC AAAATTTGCT     2040
GAAAGTTATG GGATGAAGCT AGAAAATGTC TTGTTTAATA TGGACGGAAC AATTGAATTA     2100
TATTTACCAT CGGGAGAAGT CATTAAAAAG AATATGGCAG ATTTTACAGG AGAAGCACCT     2160
CAAGGAAATG GTGAAAATAA ACCATCTGAA AATGGAAAAG TATCTACTGG AACAGTTGAG     2220
AACCAACCAA CAGAAAATAA ACCAGCAGAT TCTTTACCAG AGGCACCAAA CGAAAAACCT     2280
GTAAAACCAG AAAACTCAAC GGATAATGGA ATGTTGAATC CAGAAGGGAA TGTGGGGAGT     2340
GACCCTATGT TAGATTCAGC ATTAGAGGAA GCTCCAGCAG TAGATCCTGT ACAAGAAAAA     2400
TTAGAAAAAT TTACAGCTAG TTACGGATTA GGCTTAGATA GTGTTATATT CAATATGGAT     2460
GGAACGATTG AATTAAGATT GCCAAGTGGA GAAGTGATAA AAAAGAATTT ATTGATCTCA     2520
TAGCGTAA   (SEQ ID NO : 9)                                            2528
```

FIGURE 9

```
CAYALNQHRS QENKDNNRVS YVDGSQSSQK SENLTPDQVS QKEGIQAEQI       50
VIKITDQGYV TSHGDHYHYY NGKVPYDALF SEELLMKDPN YQLKDADIVN      100
EVKGGYIIKV DGKYYVYLKD AAHADNVRTK DEINRQKQEH VKDNEKVNSN      150
VAVARSQGRY TTNDGYVFNP ADIIEDTGNA YIVPHGGHYH YIPKSDLSAS      200
ELAAAKAHLA GKNMQPSQLS YSSTPSPSLP INPGTSHEKH EEDGYGFDAN      250
RIIAEDESGF VMSHGDHNHY FFKKDLTEEQ IKAAQKHLEE VKTSHNGLDS      300
LSSHEQDYPS NAKEMKDLDK KIEEKIAGIM KQYGVKRESI VVNKEKNAII      350
YPHGDHHHAD PIDEHKPVGI GHSHSNYELF KPEEGVAKKE GNKVYTGEEL      400
```

```
TNVVNLLKNS TFNNQNFTLA NGQKRVSFSF PPELEKKLGI NMLVKLITPD      450
GKVLEKVSGK VFGEGVGNIA NFELDQPYLP GQTFKYTIAS KDYPEVSYDG      500
TFTVPTSLAY KMASQTIFYP FHAGDTYLRV NPQFAVPKGT DALVRVFDEF      550
HGNAYLENNY KVGEIKLPIP KLNQGTTRTA GNKIPVTFMA NAYLDNQSTY      600
IVEVPILEKE NQTDKPSILP QFKRNKAQEN SKLDEKVEEP KTSEKVEKEK      650
LSETGNSTSN STLEEVPTVD PVQEKVAKFA ESYGMKLENV LFNMDGTIEL      700
YLPSGEVIKK NMADFTGEAP QGNGENKPSE NGKVSTGTVE NQPTENKPAD      750
SLPEAPNEKP VKPENSTDNG MLNPEGNVGS DPMLDSALEE APAVDPVQEK      800
LEKFTASYGL GLDSVIFNMD GTIELRLPSG EVIKKNLLIS                 840
```

(SEQ ID NO : 10)
FIGURE 10

DQGYVTSHGD HYHYYNGKVP YDALFSEELL MKDPNYQLKD ADIVNEVKGG YIIKVDGKYY VYLKDAAHAD NVRTKDEINR QKQEHVKDNE KVNS
(SEQ ID NO: 11)
FIGURE 11

GIQAEQIVIK ITDQGYVTSH GDHYHYYNGK VPYDALFSEE LL
(SEQ ID NO: 12)
FIGURE 12

TAYIVRHGDH FHYIPKSNQI GQPTLPNNSL ATPSPSLPI
(SEQ ID NO: 13)
FIGURE 13

TSNSTLEEVP TVDPVQEKVA KFAESYGMKL ENVLFN
(SEQ ID NO: 14)
FIGURE 14

MDGTIELRLP SGEVIKKNLS DFIA
(SEQ ID NO: 15)
FIGURE 15

YGLGLDSVIF NMDGTIELRL PSGEVIKKNL SDFIA
(SEQ ID NO: 16)
FIGURE 16

PALEEAPAVD PVQEKLEKFT ASYGLGLDSV IFNMDGTIEL RLPSGEVIKK NLSDFIA
(SEQ ID NO: 17)
FIGURE 17

KVEEPKTSEK VEKEKLSETG NSTSNSTLEE VPTVDPVQEK
(SEQ ID NO: 18)
FIGURE 18

MKDLDKKIEE KIAGIMKQYG VKRESIVVNK EKNAIIYPHG DHHHADPIDE HKPVGIGHSH SNYELFKPEE GVAKKEGN
(SEQ ID NO: 19)
FIGURE19

AIIYPHGDHH HADPIDEHKP VGIGHSHSNY ELFKPEEGVA KKEGNKVYTG E
(SEQ ID NO: 20)
FIGURE 20

IQVAKLAGKY TTEDGYIFDP RDITSDEGD
(SEQ ID NO: 21)
FIGURE 21

DHQDSGNTEA KGAEAIYNRV KAAKKVPLDR MPYNLQYTVE VKNGSLIIPH YDHYHNIKFE WFDEGLYEAP KGYSLEDLLA TVKYYV
(SEQ ID NO: 22)
FIGURE 22

GLYEAPKGYS LEDLLATVKY YVEHPNERPH SDNGFGNASD H
(SEQ ID NO: 23)
FIGURE 23

GLYEAPKGYSLEDLLATVKYYV
(SEQ ID NO: 163)
Figure 24

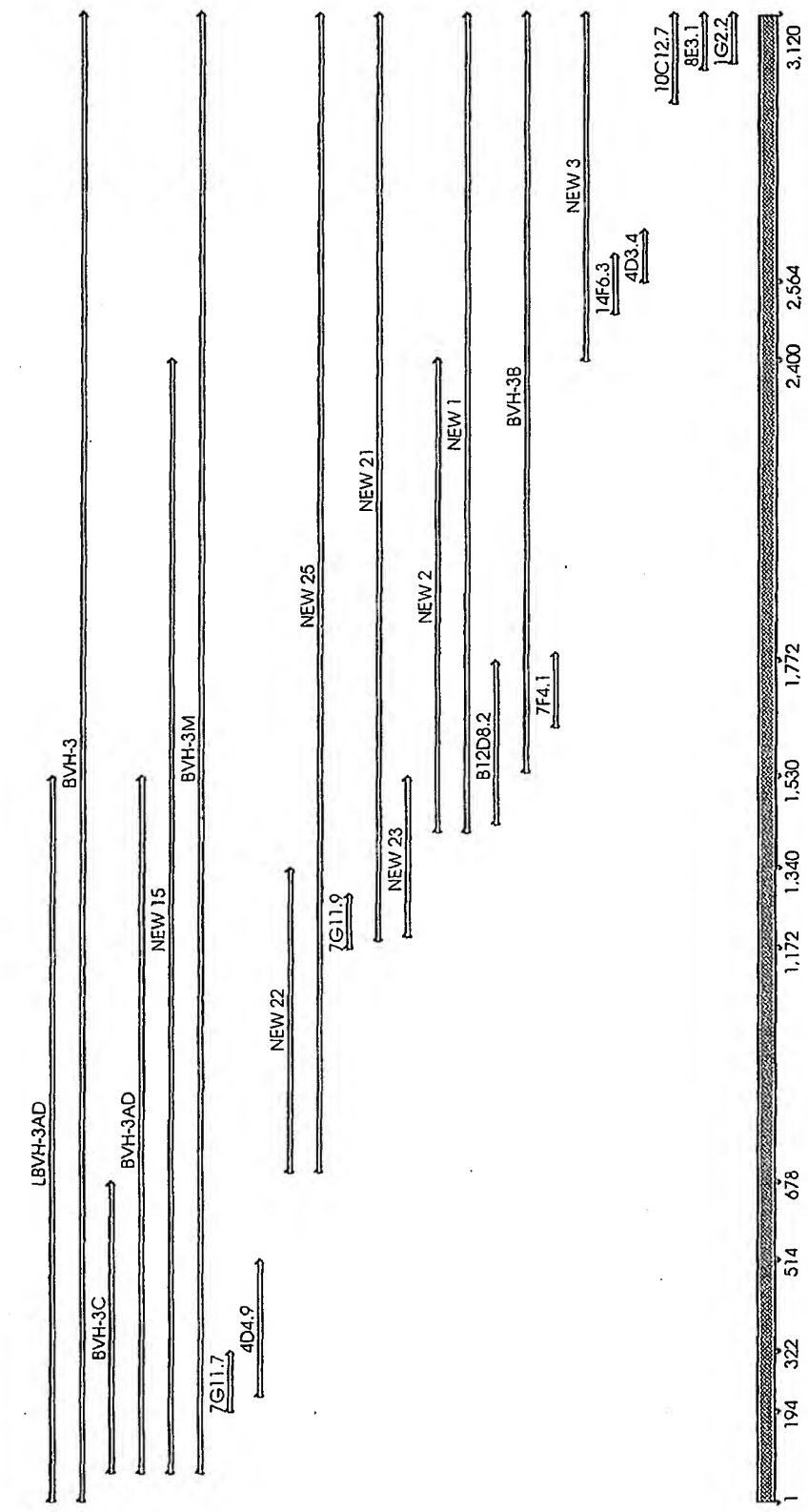

LBVH-3AD
BVH-3
BVH-3C
BVH-3AD
NEW 15
BVH-3M
7G11.7
4D4.9
NEW 22
NEW 25
7G11.9
NEW 21
NEW 23
NEW 2
NEW 1
B12D8.2
BVH-3B
7F4.1
NEW 3
14F6.3
4D3.4
10C12.7
8E3.1
1G2.2
1
194
322
514
678
1,172
1,340
1,530
1,772
2,400
2,564
3,120

FIGURE 25

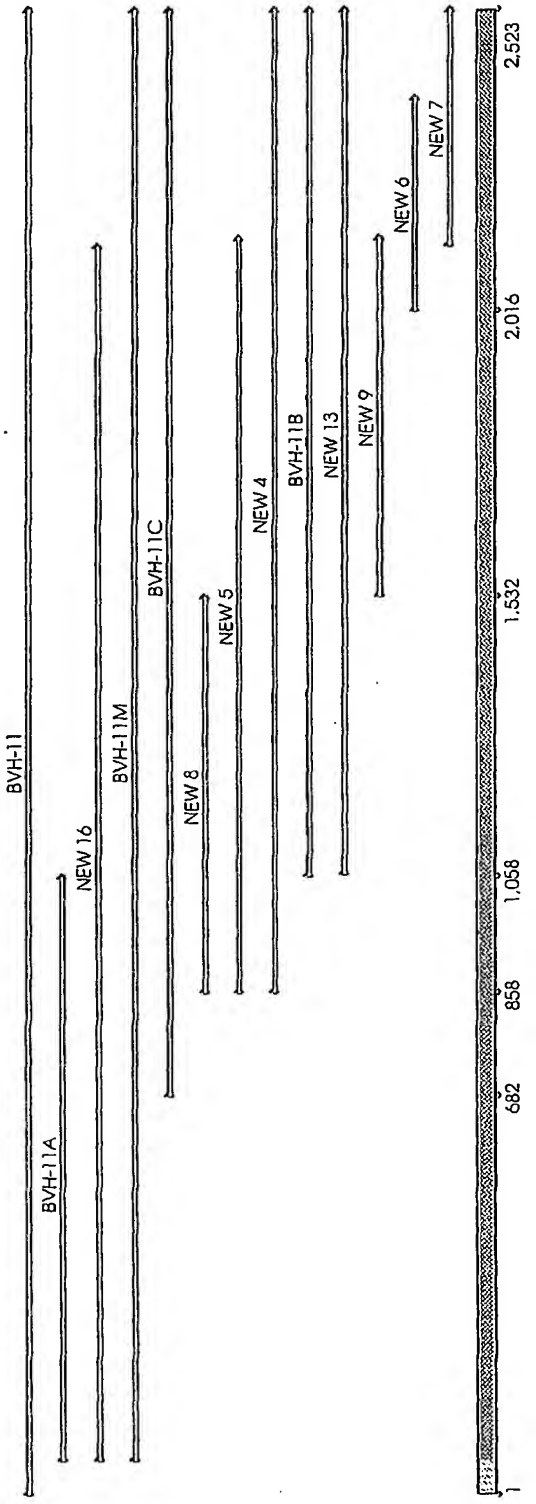

BVH-11
BVH-11A
NEW 16
BVH-11M
BVH-11C
NEW 8
NEW 5
NEW 4
BVH-11B
NEW 13
NEW 9
NEW 6
NEW 7
1
682
858
1.058
1.532
2.016
2.523

FIGURE 26

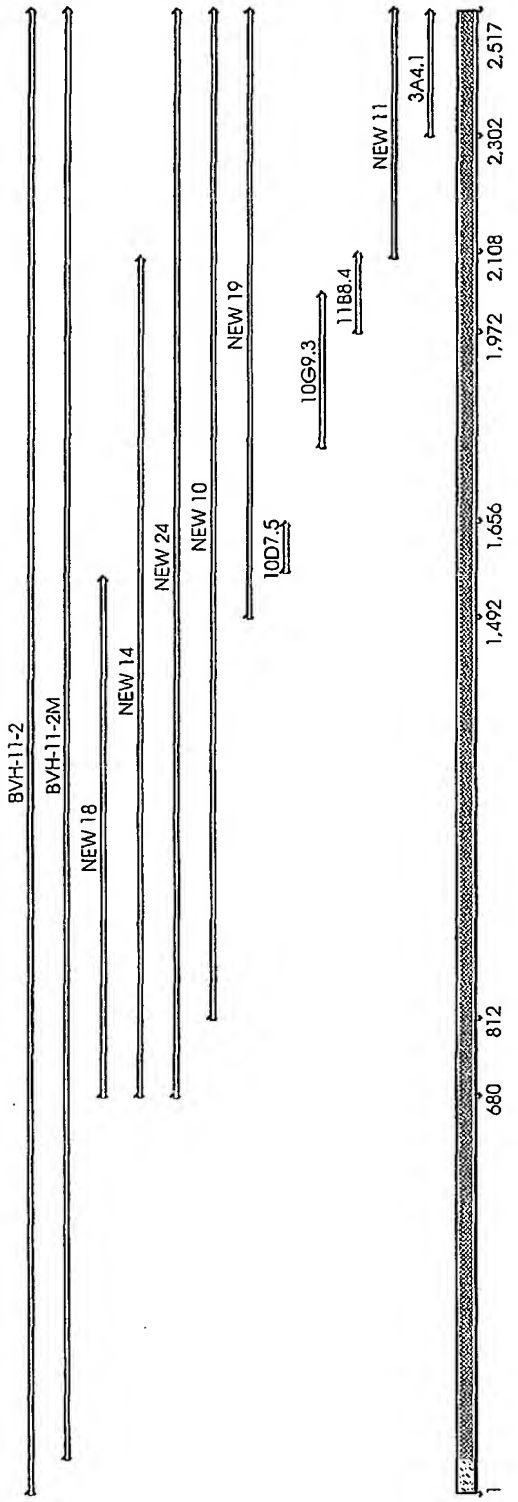

BVH-11-2
BVH-11-2M
NEW 18
NEW 14
NEW 24
NEW 10
NEW 19
10D7.5
10G9.3
1188.4
NEW 11
3A4.1
1
680
812
1,492
1,656
1,972
2,108
2,302
2,517

FIGURE 27

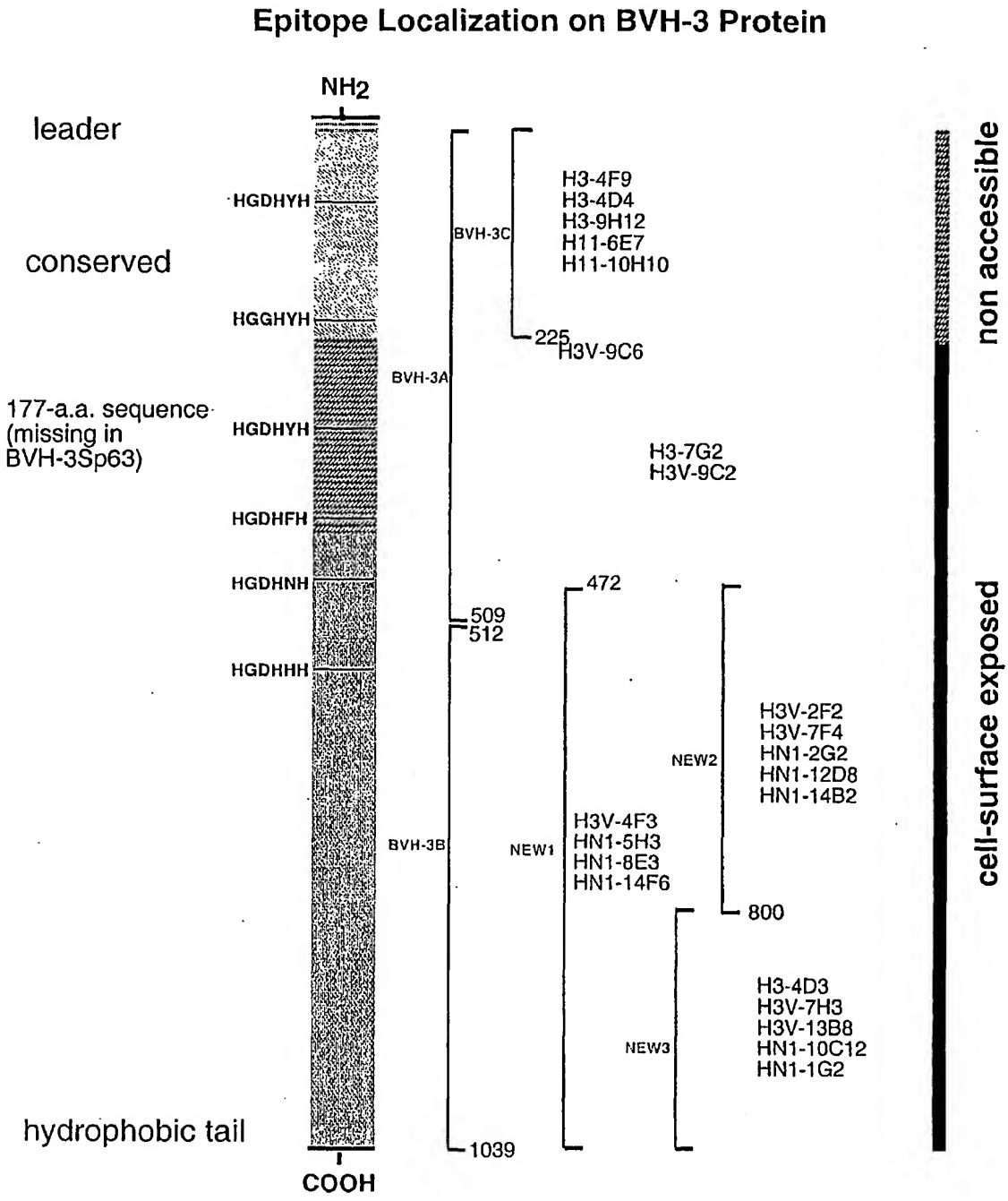
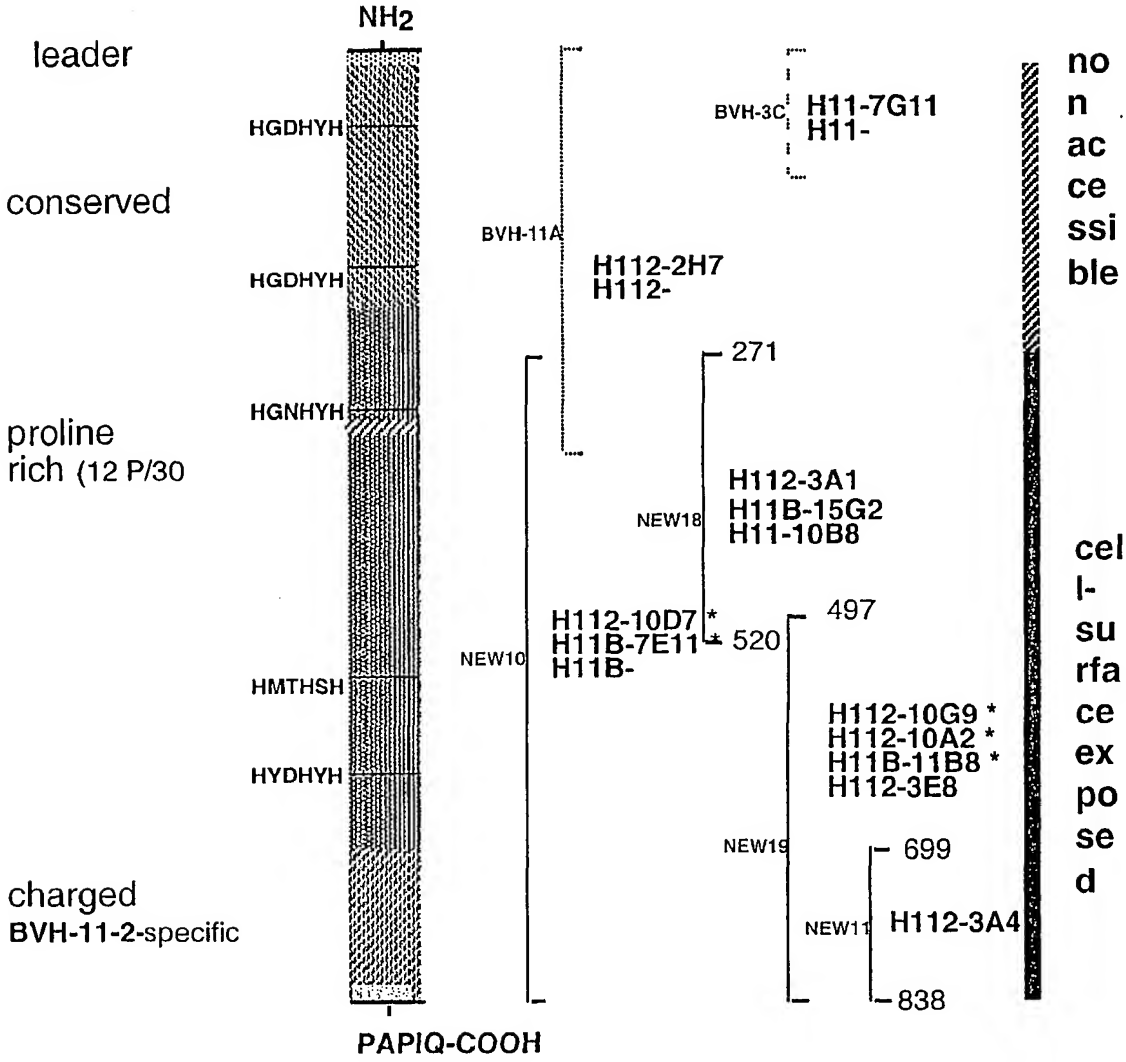

Epitope Localization on BVH-3 Protein
NH2
leader
conserved
177-a.a. sequence (missing in BVH-3Sp63)
hydrophobic tail
COOH
HGDHYH
HGGHYH
HGDHYH
HGDHFH
HGDHNH
HGDHHH
BVH-3C
BVH-3A
BVH-3B
H3-4F9
H3-4D4
H3-9H12
H11-6E7
H11-10H10
225
H3V-9C6
H3-7G2
H3V-9C2
472
509
512
NEW1
H3V-4F3
HN1-5H3
HN1-8E3
HN1-14F6
NEW2
H3V-2F2
H3V-7F4
HN1-2G2
HN1-12D8
HN1-14B2
800
NEW3
H3-4D3
H3V-7H3
H3V-13B8
HN1-10C12
HN1-1G2
1039
non accessible
cell-surface exposed

FIGURE 28

Epitope Localization on BVH-11-2 Protein



* Surface-exposed and protection-conferring Mabs

FIGURE 29

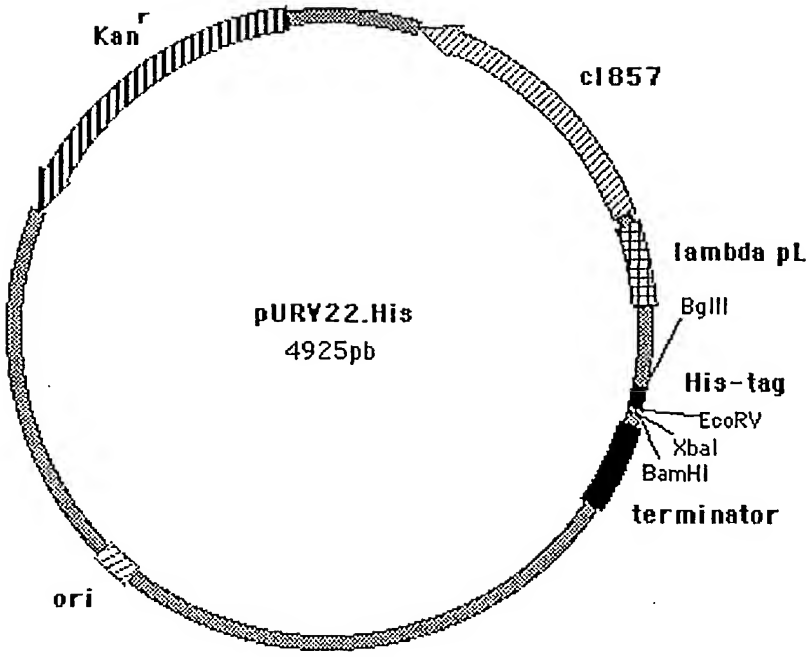

Kan r
cI857
lambda pL
BglII
His-tag
EcoRV
XbaI
BamHI
terminator
ori
pURV22_His
4925pb

FIGURE 30

```
BVH-3M      1 CAYALNQHRSQENKDNNRVSYVDGSQSSQKSENLTPDQVSQKEGIQAEQIVIKITDQGYV   60
BVH3-63     1 CAYALNQHRSQENKDNNRVSYVDGSQSSQKSENLTPDQVSQKEGIQAEQIVIKITDQGYV   60
              ************************************************************

BVH-3M     61 TSHGDHYHYYNGKVPYDALFSEELLMKDPNYQLKDADIVNEVKGGYIIKVDGKYYVYLKD  120
BVH3-63    61 TSHGDHYHYYNGKVPYDALFSEELLMKDPNYQLKDADIVNEVKGGYIIKVDGKYYVYLKD  120
              ************************************************************

BVH-3M    121 AAHADNVRTKDEINRQKQEHVKDNEKVNSNVAVARSQGRYTTNDGYVFNPADIIEDTGNA  180
BVH3-63   121 AAHADNVRTKDEINRQKQEHVKDNEKVNSNVAVARSQGRYTTNDGYVFNPADIIEDTGNA  180
              ************************************************************

BVH-3M    181 YIVPHGGHYHYIPKSDLSASELAAAKAHLAGKNMQPSQLSYSSTASDNNTQSVAKGSTSK  240
BVH3-63   181 YIVPHGGHYHYIPKSDLSASELAAAKAHLAGKNMQPSQLSYSS----------------  223
              ******************************************

BVH-3M    241 PANKSENLQSLLKELYDSPSAQRYSESDGLVFDPAKIISRTPNGVAIPHGDHYHFIPYSK  300
BVH3-63   224 ------------------------------------------------------------  223

BVH-3M    301 LSALEEKIARMVPISGTGSTVSTNAKPNEVVSSLGSLSSNPSSLTTSKELSSASDGYIFN  360
BVH3-63   224 ------------------------------------------------------------  223

BVH-3M    361 PKDIVEETATAYIVRHGDHFHYIPKSNQIGQPTLPNNSLATPSPSLPINPGTSHEKHEED  420
BVH3-63   224 ----------------------------------------TPSPSLPINPGTSHEKHEED  243
                                                       ********************

BVH-3M    421 GYGFDANRIIAEDESGFVMSHGDHNHYFFKKDLTEEQIKAAQKHLEEVKTSHNGLDSLSS  480
BVH3-63   244 GYGFDANRIIAEDESGFVMSHGDHNHYFFKKDLTEEQIKAAQKHLEEVKTSHNGLDSLSS  303
              ************************************************************

BVH-3M    481 HEQDYPGNAKEMKDLDKKIEEKIAGIMKQYGVKRESIVVNKEKNAIIYPHGDHHHADPID  540
BVH3-63   304 HEQDYPSNAKEMKDLDKKIEEKIAGIMKQYGVKRESIVVNKEKNAIIYPHGDHHHADPID  363
              ****** ***************************************************

BVH-3M    541 EHKPVGIGHSHSNYELFKPEEGVAKKEGNKVYTGEELTNVVNLLKNSTFNNQNFTLANGQ  600
BVH3-63   364 EHKPVGIGHSHSNYELFKPEEGVAKKEGNKVYTGEELTNVVNLLKNSTFNNQNFTLANGQ  423
              ************************************************************

BVH-3M    601 KRVSFSFPPELEKKLGINMLVKLITPDGKVLEKVSGKVFGEGVGNIANFELDQPYLPGQT  660
BVH3-63   424 KRVSFSFPPELEKKLGINMLVKLITPDGKVLEKVSGKVFGEGVGNIANFELDQPYLPGQT  483
```

```
                ************************************************************

BVH-3M    661 FKYTIASKDYPEVSYDGTFTVPTSLAYKMASQTIFYPFHAGDTYLRVNPQFAVPKGTDAL  720
BVH3-63   484 FKYTIASKDYPEVSYDGTFTVPTSLAYKMASQTIFYPFHAGDTYLRVNPQFAVPKGTDAL  543
                ************************************************************

BVH-3M    721 VRVFDEFHGNAYLENNYKVGEIKLPIPKLNQGTTRTAGNKIPVTFMANAYLDNQSTYIVE  780
BVH3-63   544 VRVFDEFHGNAYLENNYKVGEIKLPIPKLNQGTTRTAGNKIPVTFMANAYLDNQSTYIVE  603
                ************************************************************

BVH-3M    781 VPILEKENQTDKPSILPQFKRNKAQENSKLDEKVEEPKTSEKVEKEKLSETGNSTSNSTL  840
BVH3-63   604 VPILEKENQTDKPSILPQFKRNKAQENSKLDEKVEEPKTSEKVEKEKLSETGNSTSNSTL  663
                ************************************************************

BVH-3M    841 EEVPTVDPVQEKVAKFAESYGMKLENVLFNMDGTIELYLPSGEVIKKNMADFTGEAPQGN  900
BVH3-63   664 EEVPTVDPVQEKVAKFAESYGMKLENVLFNMDGTIELYLPSGEVIKKNMADFTGEAPQGN  723
                ************************************************************

BVH-3M    901 GENKPSENGKVSTGTVENQPTENKPADSLPEAPNEKPVKPENSTDNGMLNPEGNVGSDPM  960
BVH3-63   724 GENKPSENGKVSTGTVENQPTENKPADSLPEAPNEKPVKPENSTDNGMLNPEGNVGSDPM  783
                ************************************************************

BVH-3M    961 LDPALEEAPAVDPVQEKLEKFTASYGLGLDSVIFNMDGTIELRLPSGEVIKKNLSDFIA  1019
BVH3-63   784 LDSALEEAPAVDPVQEKLEKFTASYGLGLDSVIFNMDGTIELRLPSGEVIKKNLLIS     840
                ** ***************************************************
```

FIGURE 31

```
BVH-3       1 MKFSKKYIAAGSAVIVSLSLCAYALNQHRSQENK-DNNRVSYVDGSQSSQKSENLTPDQV   59
BVH-11      1 MKINKKYLAG-SVATLVLSVCAYELGLHQAQTVK-ENNRVSYIDGKQATQKTENLTPDEV   58
BVH-11-2    1 MKINKKYLAG-SVAVLALSVCSYELGRHQAGQVKKESNRVSYIDGDQAGQKAENLTPDEV   59
                **  ***.*  *   . **.*.* *. *..   * . *****.** *. **.******.*

BVH-3      60 SQKEGIQAEQIVIKITDQGYVTSHGDHYHYYNGKVPYDALFSEELLMKDPNYQLKDADIV  119
BVH-11     59 SKREGINAEQIVIKITDQGYVTSHGDHYHYYNGKVPYDAIISEELLMKDPNYQLKDSDIV  118
BVH-11-2   60 SKREGINAEQIVIKITDQGYVTSHGDHYHYYNGKVPYDAIISEELLMKDPNYQLKDSDIV  119
                *..***.*********************************. ***************.***

BVH-3     120 NEVKGGYIIKVDGKYYVYLKDAAHADNVRTKDEINRQKQEHVKDNE----KVNSNVAVAR  175
BVH-11    119 NEIKGGYVIKVNGKYYVYLKDAAHADNVRTKEEINRQKQEHSQHREGGTSANDGAVAFAR  178
BVH-11-2  120 NEIKGGYVIKVDGKYYVYLKDAAHADNIRTKEEIKRQKQEHSHNHN---SRADNAVAAAR  176
                **.****.*** ***************.***.** ******  .            ** **
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```
BVH-3      176 SQGRYTTNDGYVFNPADIIEDTGNAYIVPHGGHYHYIPKSDLSASELAAAKAHLAGKNMQ  235
BVH-11     179 SQGRYTTDDGYIFNASDIIEDTGDAYIVPHGDHYHYIPKNELSASELAAAEAFLSGRENL  238
BVH-11-2   177 AQGRYTTDDGYIFNASDIIEDTGDAYIVPHGDHYHYIPKNELSASELAAAEAYWNGKQ--  234
                .****** ***.** .******* ******* ******* .********* *   *.

BVH-3      236 PSQLSYSSTASDNNTQSVAKGSTSKP------A-N---------KSENLQSLLKELYDSP  279
BVH-11     239 SNLRTYRRQNSDNTPRTNWVPSVSNPGTTNTNTSNNSNTNSQASQSNDIDSLLKQLYKLP  298
BVH-11-2   235 -GSRPSSSSSYNANPVQPRLSENHNLTVTPTYHQN---------QGENISSLLRELYAKP  284
                              .                    *         .   . ***..**  *

BVH-3      280 SAQRYSESDGLVFDPAKIISRTPNGVAIPHGDHYHFIPYSKLSALEEKIARMVPISGTGS  339
BVH-11     299 LSQRHVESDGLIFDPAQITSRTARGVAVPHGNHYHFIPYEQMSELEKRIARIIPLRYRSN  358
BVH-11-2   285 LSERHVESDGLIFDPAQITSRTARGVAVPHGNHYHFIPYEQMSELEKRIARIIPLRYRSN  344
                 ..*  *****.****.* ***  ***.*** ******* ..* ** .***..*.

BVH-3      340 TVSTNAKPNEVVSSLGSLSSNPSSLTTSKELSSASDGYIFNPKDIVEETATAYIVRHGDH  399
BVH-11     359 HWVPDSRP-EEPSPQPTPEPSPS-PQPAPNPQPAPS----NP--IDEKLVKEAVRKVGDG  410
BVH-11-2   345 HWVPDSRP-EQPSPQSTPEPSPS-LQPAPNPQPAPS----NP--IDEKLVKEAVRKVGDG  396
                    ..* *  *   .    **    .     *      **  * *      . . **

BVH-3      400 FHYIPKSNQIGQPTLPNNSLATPSPSLPINPGTSHEKHEEDGYGFDANRIIAEDESGFVM  459
BVH-11     411 YVFEE------------NGVSRYIP--------AKNLSAETAAGIDSKLAKQESLS----  446
BVH-11-2   397 YVFEE------------NGVSRYIP--------AKDLSAETAAGIDSKLAKQESLS----  432
                . .              * ..   *        ..    *   * *.     *  *

BVH-3      460 SHGDHNHYFFKKDLTEEQIKAAQKHLEEVKTSHNGLDSLSSHEQDYPGNAKEMKDLDKKI  519
BVH-11     447 ----HKLGAKKTDLPSSDREFYNKAYDLLARIHQDLLDNKGRQVDFEALDNLLERLKDVS  502
BVH-11-2   433 ----HKLGAKKTDLPSSDREFYNKAYDLLARIHQDLLDNKGRQVDFEVLDNLLERLKDVS  488
                    *     * **        .*  . .   *. *     .. *.      .  *

BVH-3      520 EEKIAGIMKQYGVKRESIVVNKEKNAIIYPHGDHHHADPIDEHKPVGIGHSHSNYELFKP  579
BVH-11     503 SDKVKLVDDILAFLAP--IRHPER--LGKPNAQITYTDDEIQVAKLAGKYTTEDGYIFDP  558
BVH-11-2   489 SDKVKLVDDILAFLAP--IRHPER--LGKPNAQITYTDDEIQVAKLAGKYTTEDGYIFDP  544
                .*.  .          . . *.  .  *.     .*   .  .    .  .  .* *

BVH-3      580 EEGVAKKEGNKVYTGEELTNVVNLLKNSTFNNQNFTLANGQKRVSFSFPPELEKKLGINM  639
BVH-11     559 RD-ITSDEGD-AYVTPHMTHSHWIKKDS-LSEAERAAAQAYAKEKGLTPPSTDHQD----  611
BVH-11-2   545 RD-ITSDEGD-AYVTPHMTHSHWIKKDS-LSEAERAAAQAYAKEKGLTPPSTDHQD----  597
                . ..  **   *    .*.   . * *       . *.   .     **  ...

BVH-3      640 LVKLITPDGKVLEKVSGKVFGEGVGNIANFELDQPYLPGQTFKYTIASKDYPEVSYDGTF  699
BVH-11     612 ---------------SGNTEAKGAEAIYNRVKAAKKVPLDRMPYNLQ---YTVEVKNGSL  653
BVH-11-2   598 ---------------SGNTEAKGAEAIYNRVKAAKKVPLDRMPYNLQ---YTVEVKNGSL  639
                             **     *   * *       .*   .  *..    *      *.
```

```
BVH-3      700 TVPTSLAYKMASQTIFYPFHAGDTYLRVNPQFAVPKGTDALVRVFDEFHGNAYLENNYKV  759
BVH-11     654 IIP---HYDHYHNIKFEWFDEG--------LYEAPKG---------------YTLEDLLAT  688
BVH-11-2   640 IIP---HYDHYHNIKFEWFDEG--------LYEAPKG---------------YSLEDLLAT  674
                .*    *    .  *  *  *         .  ***                **

BVH-3      760 GEIKLPIPKLNQGTTRTAGNKIPVTFMANAYLDNQSTYIVEVPILEKENQTDKPSILPQF  819
BVH-11     689 VKYYVEHPNERPHSDNGFGN-------ASDHVQRN-----------KNGQADTN------  724
BVH-11-2   675 VKYYVEHPNERPHSDNGFGN-------ASDHVRKN-----------KADQDSKP------  710
                    .  *     .    **       *   .  .           *  *

BVH-3      820 KRNKAQENSKLDEKVEEPKTSEKVEKEKLSETGNSTSNSTLEEVPTVDPVQEKVAKFAES  879
BVH-11     725 ------QTEKPSEEKPQTEKPE---EE-------------------------------  742
BVH-11-2   711 ------DEDKEHDEVSEPTHPESDEKE-----------------------------------  731
                     *  .   .    *    *

BVH-3      880 YGMKLENVLFNMDGTIELYLPSGEVIKKNMADFTGEAPQGNGENKPSENGKVSTGTVENQ  939
BVH-11     743 -------------------------------TPREEKPQSE---KPES----------PK  758
BVH-11-2   732 -------------------------------NHAGLNPSADNLYKPSTD---------TE  751
                                                 *      **

BVH-3      940 PTENKPADSLPEAPNEKPVKPENSTDNGMLNPEGNVGSDPMLDPALEEAPAVDPVQEKLE  999
BVH-11     759 PTEEPEEESPEES--EEPQVETEKVEEKLREAEDLLGK--IQDPIIKSN-----AKETLT  809
BVH-11-2   752 ETEEEAEDTTDEA--EIPQVENSVINAKIADAEALLEK--VTDPSIRQN-----AMETLT  802
                    **    ..  *.  * *          .   *  .    . ** .          * *

BVH-3     1000 KFTASYGLGLDSVIFNMDGTIELRLPSGEVIKKNLSDFIA  1039
BVH-11     810 GLKNNLLFGTQ-----DNNTIMAEAEKLLALLKESK       840
BVH-11-2   803 GLKSSLLLGTK-----DNNTISAEVDSLLALLKESQPAPIQ  838
                      *         .**         . *
```

FIGURE 32

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  1 ATGCAAATTA CCTACACTGA TGATGAGATT CAGGTAGCCA AGTTGGCAGG CAAGTACACA
 61 ACAGAAGACG GTTATATCTT TGATACTAGT TGGATTAAAA AAGATAGTTT GTCTGAAGCT
121 GAGAGAGCGG CAGCCCAGGC TTATGCTAAA GAGAAAGGTT TGACCCCTCC TTCGACAGAC
181 CACCAGGATT CAGGAAATAC TGAGGCAAAA GGAGCAGAAG CTATCTACAA CCGCGTGAAA
241 GCAGCTAAGA AGGTGCCACT TGATCGTATG CCTTACAATC TTCAGTATAC TGTAGAAGTC
301 AAAAACGGTA GTTTAATCAT ACCTCATTAT GACCATTACC ATAACATCAA ATTTGAGTGG
361 TTTGACGAAG GCCTTTATGA GGCACCTAAG GGGTATAGTC TTGAGGATCT TTTGGCGACT
421 GTCAAGTACT ATGTCGAACC GCGGAACGCT AGTGACCATG TTCGTAAAAA TAAGGCAGAC
481 CAAGATAGTA AACCTGATGA AGATAAGGAA CATGATGAAG TAAGTGAGCC AACTCACCCT
541 GAATCTGATG AAAAAGAGAA TCACGCTGGT TTAAATCCTT CAGCAGATAA TCTTTATAAA
601 CCAAGCACTG ATACGGAAGA GACAGAGGAA GAAGCTGAAG ATACCACAGA TGAGGCTGAA
661 ATTCCTGGTA CCCCTAGTAT TAGACAAAAT GCTATGGAGA CATTGACTGG TCTAAAAAGT
721 AGTCTTCTTC TCGGAACGAA AGATAATAAC ACTATTTCAG CAGAAGTAGA TAGTCTCTTG
781 GCTTTGTTAA AAGAAAGTCA ACCGGCTCCT ATACAGTAG  (SEQ ID NO: 257)
```

FIGURE 33

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  1 MQITYTDDEI QVAKLAGKYT TEDGYIFDTS WIKKDSLSEA ERAAAQAYAK EKGLTPPSTD
 61 HQDSGNTEAK GAEAIYNRVK AAKKVPLDRM PYNLQYTVEV KNGSLIIPHY DHYHNIKFEW
121 FDEGLYEAPK GYSLEDLLAT VKYYVEPRNA SDHVRKNKAD QDSKPDEDKE HDEVSEPTHP
181 ESDEKENHAG LNPSADNLYK PSTDTEETEE EAEDTTDEAE IPGTPSIRQN AMETLTGLKS
241 SLLLGTKDNN TISAEVDSLL ALLKESQPAP IQ   (SEQ ID NO : 258)
```

FIGURE 34